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Health Assessment Document for Polycyclic Organic Matter

Syracuse Research Corp., NY

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16. ABSTRACT

This document responds to Section 122 of the Clean Air Act as Amended August 1977, which requires the Administrator to decide whether atmospheric emissions of polycyclic organic matter (POM) potentially endanger public health.

This document reviews POM data on chemical and physical properties, atmospheric forms, atmospheric fate and transport, measurement techniques, ambient levels, toxicology, occupational health, and epidemiology.

Polycyclic aromatic hydrocarbons (PAH's), such as the carcinogen benzo[a]pyrene (BaP), and their neutral nitrogen analogs are the two POM chemical groups occurring most frequently in ambient air. The major environmental sources of POM's appear to be the combustion or pyrolysis of materials containing carbon and hydrogen.

There is general agreement that POM compounds are associated with suspended particulate matter from both mobile and stationary sources, principally respirable particles. Available monitoring data suggest that many POM compounds associated with particulate matter probably are stable in ambient air for several days.

The major health concern over exposure to POM's is their carcinogenicity. POM's gain ready access to the body's circulation either by inhalation, ingestion, or skin contact. Although it cannot be stated unequivocally that any POM's are human carcinogens, several of these compounds are among the more potent animal carcinogens known.

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combustion	coal tar	suspended particulate matter	06F
pyrolysis	coal gas	mobile sources	06J
carcinogens	urologic diseases	stationary sources	06P
nitrogen heterocyclic	compounds	aza arenes	06T
bituminous coal	pitch (material)	imino arenes	07C
respiratory diseases	public health	cigarette smoking	

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by

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PREFACE

This document was written in response to Section 122 of the Clean Air Act Amendments of 1977, P.L. 95-95, as noted under 42 U.S.C. Section 7548. The purpose of this report is to decide whether or not polycyclic organic matter (POM) emissions into the ambient air cause or contribute to air pollution which may reasonably be anticipated to endanger public health.

The document constitutes an in-depth review of the original published literature and the current scientific position with regard to knowledge of polycyclic organic matter. Chemical and physical properties, atmosphere forms, fate and transport, measurement techniques, ambient levels, toxicology and occupational and epidemiological data on POM's have been reviewed.

The Agency is pleased to acknowledge the efforts of all persons and groups who have participated in preparing this document.

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ABSTRACT

This document was prepared in response to the Clean Air Act Amendments of 1977 and constitutes an in-depth scientific review and assessment of the literature on polycyclic organic matter relative to the health and ecological effects associated with exposure to these complex organic molecules, either as pure compounds or as POM-containing effluents from industralized sources. It does not cite every published article relating to POM since a Multimedia Health Assessment Document for POM, J. Environ. Pathol. Toxicol. (in press, 1980), has been prepared for EPA by Syracuse Research Corporation and covers exposure from air, food, and water sources.

In addition to the chapters devoted to the health and ecological effects associated with exposure to POM, there are additional topics discussed which are necessary to support these effects from a qualitative and quantitative viewpoint. These include separate chapters which discuss chemical and physical properties, atmospheric forms, atmospheric fate and transport, measurement techniques, and ambient atmospheric levels.

TABLE OF CONTENTS

Page

1. EXECUTIVE SUMMARY........ 1-1
   1.1 DEFINITION AND FORMATION........ 1-1
   1.2 ANALYTICAL METHODS........ 1-2
   1.3 AMBIENT CONCENTRATIONS........ 1-3
   1.4 HEALTH AND ECOLOGICAL EFFECTS........ 1-4

2. INTRODUCTION........ 2-1
   2.1 REFERENCES........ 2-3

3. PHYSICAL AND CHEMICAL DATA........ 3-1
   3.1 DEFINITION AND FORMATION........ 3-1
   3.2 STRUCTURE AND PROPERTIES........ 3-4
      3.2.1 Chemical Structure........ 3-4
      3.2.2 Physical Properties........ 3-27
      3.2.3 Atmospheric Forms of POM........ 3-28
   3.3 ATMOSPHERIC CHEMISTRY........ 3-35
      3.3.1 Photooxidation........ 3-35
      3.3.2 Oxidation and Nitration........ 3-41
      3.3.3 Atmospheric Stability........ 3-45
   3.4 SUMMARY AND CONCLUSIONS........ 3-45
   3.5 REFERENCES........ 3-48

4. SAMPLING AND ANALYTICAL METHODS FOR POM........ 4-1
   4.1 INTRODUCTION........ 4-1
   4.2 SAMPLING........ 4-3
      4.2.1 Mobile Sources........ 4-4
      4.2.2 Tobacco Smoke........ 4-6
      4.2.3 Stationary Sources........ 4-6
         4.2.3.1 Emissions from stacks........ 4-6
         4.2.3.2 Fugitive emissions from road-way dust........ 4-10
      4.2.4 Ambient Air........ 4-11
         4.2.4.1 Collection of total air particulate matter........ 4-12
         4.2.4.2 Distribution of particle size. 4-14
   4.3 DESORPTION OF POM FROM COLLECTION MEDIA........ 4-16
      4.3.1 Extraction........ 4-16
         4.3.1.1 Solvent extraction........ 4-17
         4.3.1.2 Mechanical disruption........ 4-19
         4.3.1.3 Hydrofluoric acid dissolution........ 4-19
      4.3.2 Thermal Stripping........ 4-21
      4.3.3 Vacuum Sublimation........ 4-22
   4.4 CLEAN-UP AND SEPARATION........ 4-23
      4.4.1 Solvent Partitioning........ 4-24
         4.4.1.1 Methanol-water........ 4-24
         4.4.1.2 Tetramethyl uric acid in methanol........ 4-25
         4.4.1.3 Acetonitrile........ 4-25
         4.4.1.4 Dimethyl formamide........ 4-25
         4.4.1.5 Nitromethane........ 4-25
         4.4.1.6 Dimethyl sulfoxide........ 4-25

Page

4.4.2 Column Chromatography 4-27
4.4.2.1 Alumina 4-28
4.4.2.2 Silica gel 4-28
4.4.2.3 Florisil 4-29
4.4.2.4 Cellulose acetate 4-30
4.4.2.5 Gel filtration 4-30
4.4.3 Paper Chromatography 4-31
4.4.4 Paper and Thin-layer Electrophoresis 4-31
4.4.5 Thin-layer Chromatography 4-31
4.4.6 Gas Chromatography 4-34
4.4.6.1 Packed column 4-34
4.4.6.2 Capillary column 4-35
4.4.7 High Pressure Liquid Chromatography 4-36
4.5 DETECTION 4-39
4.5.1 Flame Ionization and Electron Capture 4-39
4.5.2 U.V. Absorption 4-40
4.5.3 Luminescence Analysis 4-40
4.5.3.1 U.V. excited luminescence emission 4-40
4.5.3.2 Shpol'skii effect 4-42
4.5.3.3 X-ray excited optical luminescence (XEOL) 4-43
4.5.3.4 Sensitized fluorescence 4-44
4.5.3.5 Synchronous luminescence spectroscopy 4-44
4.5.4 Mass Spectrometry 4-45
4.5.5 Other Techniques 4-46
4.6 ANALYTICAL METHOD USED BY EPA FOR NASN MONITORED SAMPLES 4-46
4.7 DIFFICULTIES IN POM MONITORING AND ANALYSIS 4-48
4.7.1 Sampling Errors 4-48
4.7.1.1 Nonisokinetic sampling 4-48
4.7.1.2 Non-incorporation of sampling efficiency 4-49
4.7.1.3 Evaporative losses and reactive conversion 4-49
4.7.2 Losses During Transportation and Storage 4-51
4.7.3 Errors in Analytical Procedures 4-53
4.7.3.1 Losses during desorption process 4-53
4.7.3.2 Column and thin-layer chromatographic and storage losses 4-53
4.7.3.3 Losses in evaporative concentration step 4-53
4.7.3.4 Errors due to incomplete resolution 4-53
4.7.4 Uncertainty in the Number of Parameters to be Monitored 4-55
4.8 ECONOMIC CONSIDERATION IN THE SELECTION OF THE ANALYTICAL TECHNIQUE 4-56
4.9 SUMMARY AND CONCLUSIONS 4-59
4.10 REFERENCES 4-63
5. AMBIENT LEVELS 5-1
5.1 AMBIENT ATMOSPHERIC LEVELS 5-1
5.2 SUMMARY AND CONCLUSIONS 5-39
5.3 REFERENCES 5-42

Page

6. HEALTH EFFECTS 6-1
  6.1 PHARMACOKINETICS 6-1
    6.1.1 Absorption 6-1
    6.1.2 Distribution 6-3
    6.1.3 Elimination 6-14
  6.2 METABOLISM 6-28
    6.2.1 Metabolism of POM in Animals 6-29
    6.2.2 Metabolic Bioactivation of Benzo[a]pyrene 6-37
    6.2.3 Tissue-Specific Metabolism of POM 6-40
    6.2.4 Comparative Metabolism of POM in Animals and Man 6-50
    6.2.5 Actions of Enzyme Inducers 6-54
    6.2.6 Intracellular Binding of POM 6-60
  6.3 TOXICOLOGY 6-66
    6.3.1 Acute Exposures 6-67
    6.3.2 Subchronic and Chronic Exposures 6-70
    6.3.3 Effects on the Immune Systems 6-73
  6.4 CARCINOGENESIS 6-75
    6.4.1 Structure-activity relationships 6-78
    6.4.2 Chemical Reactivity and Carcinogenicity 6-83
    6.4.3 Skin Carcinogenesis and Induction of Local Sarcomas 6-85
      6.4.3.1 Benzo[a]pyrene 6-85
      6.4.3.2 Benz[a]anthracene 6-92
      6.4.3.3 7,12-Dimethylbenz[a]anthracene 6-92
      6.4.3.4 3-Methylcholanthrene 6-97
      6.4.3.5 Dibenz[a,h]anthracene 6-98
      6.4.3.6 Dibenzo[a,h]pyrene 6-99
      6.4.3.7 Dibenzo[a,i]pyrene 6-100
      6.4.3.8 Dibenzo[c,g]carbazole 6-101
      6.4.3.9 Benzo[c]phenanthrene 6-101
      6.4.3.10 Benzo[b]fluoranthene 6-102
      6.4.3.11 Benzo[j]fluoranthene 6-103
    6.4.4 Respiratory Tract Carcinogenesis 6-103
    6.4.5 Carcinogenesis in Newborn Mice 6-111
    6.4.6 Tumors in Other Tissues 6-113
      6.4.6.1 Oral exposure to POM 6-115
    6.4.7 Carcinogenicity of POM Mixtures 6-119
    6.4.8 *In Vitro* Carcinogenesis Studies 6-126
    6.4.9 Dose-Response Models in Carcinogenesis 6-133
    6.4.10 Synergistic and Antagonistic Interactive Effects 6-140
  6.5 MUTAGENESIS 6-150
    6.5.1 *In Vitro* Studies 6-150
      6.5.1.1 Microbial systems 6-150
      6.5.1.2 Somatic cells in culture 6-156
    6.5.2 *In Vivo* Studies 6-164
      6.5.2.1 Effects in somatic tissues 6-164
      6.5.2.2 Effects in germinal tissues 6-166
    6.5.3 Correlation of Mutagenicity with Carcinogenicity 6-170
  6.6 REPRODUCTION AND TERATOLOGY 6-178
  6.7 HUMAN STUDIES 6-186
    6.7.1 Occupational Studies 6-187
    6.7.2 Community Studies 6-202

Page

6.7.2.1 Urban-rural comparisons........................ 6-204
6.7.2.2 Migrant studies................................ 6-209
6.7.2.3 Regression analyses............................ 6-209
6.7.2.4 Retrospective and prospective analyses.......... 6-219
6.8 ECOLOGICAL EFFECTS.. 6-223
6.8.1 Effects on Microorganisms and Algae.................... 6-223
6.8.2 Effects on Aquatic Organisms and Amphibians.............. 6-228
6.9 SUMMARY AND CONCLUSIONS... 6-232
6.9.1 Absorption, Distribution, and Excretion.................. 6-232
6.9.2 Metabolism and Metabolic Activation..................... 6-233
6.9.3 Toxicology... 6-237
6.9.4 Mutagenesis.. 6-238
6.9.5 Carcinogenesis... 6-240
6.9.6 Reproduction and Teratology.............................. 6-243
6.9.7 Human Studies.. 6-243
6.9.8 Ecological Effects....................................... 6-245
6.10 REFERENCES... 6-246

LIST OF FIGURES

Figure Page

3-1 Mechanism of benzo[a]pyrene formation 3-3
3-2 Accepted nomenclature for benzo[a]pyrene 3-6
3-3 Absorption specturm of benzo[a]pyrene in ethanol 3-29
3-4 Size distribution of particles containing benzo[a]pyrene 3-31
3-5 Percent mass of benzo[a]pyrene in relation to stage number for fine sampling sites in Toronto, Canada 3-33

5-1 Composite quarterly averages for BaP at 32 urban and 19 nonurban NASN stations 5-4
5-2 Benzo[a]pyrene--seasonality and trends (1966 to 1975) in the 50th and 90th percentiles for 34 NASN urban sites 5-19

6-1 Clearance of radioactivity derived from [^{3}H]3,4-benzopyrene in the lung 6-21
6-2 Clearance of radioactivity derived from [^{3}H]3,4-benzopyrene in liver 6-23
6-3 Excretion of radioactivity dervied from [^{3}H]3,4-benzopyrene in feces 6-24
6-4 Excretion of radioactivity derived from [^{3}H]3,4-benzopyrene in urine 6-25
6-5 Clearance of radioactivity derived from [^{3}H]3,4-benzopyrene in kidneys 6-26
6-6 Blood level of radioactivity derived from [^{3}H]3,4-benzopyrene 6-27
6-7 Enzymatic pathways involved in the activation and detoxification of BaP 6-30
6-8 Oxidative metabolites of benzo[a]pyrene 6-32
6-9 Probable metabolic pathways of 7-methylbenz[a]anthracene 6-36
6-10 Stereochemical course of metabolism of (+) and (-)-BaP 7,8-dihydrodiol 6-39
6-11 Comparative metabolism of benzo[a]pyrene by lung microsomes from rat, rhesus monkey, and human........ 6-52
6-12 Log-log plot of the cumulative incidence of skin carcinomas per mouse versus time........ 6-86
6-13 Incidence of cancer per rat after weekly topical application of either 20, 100, or 50 µg DMBA in 1.0 ml acetone........ 6-95
6-14 Respiratory nodule incicence in hamsters given BaP by intratracheal instillation........ 6-108
6-15 Respiratory nodule incidence in hamsters given BaP by intratracheal instillation with and without Fe_2O_3........ 6-109
6-16 The inhibiting effect of hydrocarbons found in polluted urban air and cigarette smoke in concentrations approximating those occurring naturally when injected in combination with BaP........ 6-142

LIST OF TABLES

Table Page

3-1 Polycyclic aromatic hydrocarbons 3-8
3-2 Aza and imino arenes 3-19
3-3 Miscellaneous polycyclic organic matter 3-26
3-4 Percent destruction of polycyclic aromatic hydrocarbons under atmospheric conditions 3-39
3-5 The half lives of BaP, BbF, and BkF under various reaction conditions 3-42
3-6 Comparison of PAH content of air solids before and after storage 3-44
4-1 Recovery of POM from adsorbent sampler method 4-8
4-2 Amounts of POMs collected on glass-fiber filter and polyurethane foam 4-15
4-3 Comparison of the extraction yield of POM with different solvents 4-18
4-4 Comparison of HF extraction with Soxhlet extraction 4-20
4-5 Efficiencies of solvent-partition purification procedure 4-26
4-6 Recovery of PAH by linear elution adsorption chromatography 4-29
4-7 Recovery of PAH by Sephadex LH-20 chromatography 4-30
4-8 Recovery of various PAH during thin-layer chromatography 4-32
4-9 Amounts of aza-heterocyclic compounds in a coal-tar-pitch polluted air sample separated by TLC 4-33
4-10 HPLC conditions and separation mechanisms used for PNA analysis 4-37
4-11 Percentage losses of POM from filter paper due to variation of storage time 4-52
4-12 Comparative cost, throughput, and other relevant data for four commonly used analytical methods 4-57
5-1 Influence of occupational and other factors upon benzo[a]pyrene exposure 5-2
5-2 Annual average ambient benzo[a]pyrene concentrations at NASN urban stations 5-5
5-3 Annual average ambient benzo[a]pyrene concentrations at NASN nonurban stations 5-11
5-4 Annual average ambient benzo[a]pyrene concentrations at NASN urban stations 5-13
5-5 Polycyclic aromatic compounds in the air of selected cities 5-16
5-6 Annual average concentration of PAH compounds in the air over greater Birmingham, Alabama, 1964 and 1965 5-17
5-7 Concentration of polycyclic aromatic hydrocarbons in Norway aerosols 5-25
5-8 Concentration of ten polycyclic aromatic hydrocarbons in Ontario city air 5-26
5-9 Polycyclic organic matter in ambient air 5-27
6-1 Distribution and rate of elimination of radioactivity after intravenous injection of ^{14}C-benzo[a]pyrene into rats 6-5
6-2 Distribution and rate of elimination of radioactivity after intratracheal instillation of ^{14}C-benzo[a]pyrene into rats 6-6
6-3 Effect of molecular structure on hydrocarbon localization in mammary gland and fat 6-7
6-4 Levels of orally administered 3-methylcholanthrene in tissues of 50-day-old virgin female rats 6-9

Table Page

6-5 Levels of orally administered 3-methylcholanthrene in breast and fat of virgin rats and in milk, breast, and fat of lactating rats.......... 6-10
6-6 Distribution and elimination of radioactivity after instillation of ^{3}H-dibenzo[c,g]carbazole in Syrian hamsters.......... 6-11
6-7 Concentration (μg/g) of DMBA, BaP, and MCA in tissues of 21-day fetuses, their placentas, and the liver of pregnant rats 3 hours after administration of the compounds in a dose of 200 mg/kg by gastric tube.......... 6-13
6-8 Distribution of radioactivity in rats with biliary fistulas after intravenous injection of ^{14}C-benzo[a]pyrene in plasma solution... 6-16
6-9 Amounts of B(a)P detected in hamster lungs following intratracheal instillation.......... 6-19
6-10 BaP-hydroxylase activity in different organs of the rat after methylcholanthrene and phenobarbitone pretreatment.......... 6-42
6-11 Summary of major organic solvent-soluble and water-soluble metabolites following the short-term tracheal organ culture of BP or its metabolites.......... 6-44
6-12 Comparison of benzo[a]pyrene metabolite patterns in liver and intestinal microsomes from MC-treated rats.......... 6-47
6-13 Activation of benzo[a]pyrene by colon and liver S9 fractions....... 6-49
6-14 Metabolite percentages of BP metabolites from rat, rhesus, and human lung microsomal assays.......... 6-53
6-15 Carcinogenic compounds in descending order of potency.......... 6-77
6-16 Skin tumors in mice treated with benzo[a]pyrene and derivatives.... 6-88
6-17 Summary of the skin tumor initiating activities of benzo[a]pyrene and its metabolites.......... 6-91
6-18 Induction of sarcoma by benzo[a]pyrene.......... 6-93
6-19 Comparative carcinogenicity of polycyclic hydrocarbons and related compounds, measured by induction of lung tumors (LT) in strain A mice.......... 6-105
6-20 Induction of respiratory tract tumors in Syrian golden hamsters by intratracheal instillation of POM.......... 6-106
6-21 Induction of respiratory tract tumors in rats and mice.......... 6-110
6-22 Carcinogenicity of POM by oral administration to various mammals... 6-116
6-23 Gastric tumors in mice fed benzo[a]pyrene.......... 6-118
6-24 Relationship of leukemia to lung and stomach tumors in 108 mice fed benzo[a]pyrene.......... 6-120
6-25 Classification of test groups.......... 6-122
6-26 Tumor incidence resulting, by the end of the 114th week, from a single subcutaneous application of test substances.......... 6-123
6-27 Doses (μg) applied in dermal administration experiments, in relation to benzo[a]pyrene.......... 6-124
6-28 Findings at the site of application of PAH to mouse skin.......... 6-125
6-29 Hamster embryo cell transformation produced by several polycyclic hydrocarbons and their derivatives.......... 6-129
6-30 Effect of antioxidants on benzo[a]pyrene metabolism as assayed by *Salmonella typhimurium* (strain TA98) mutagenicity in the presence of rat liver microsomal fraction and NADPH.......... 6-146
6-31 Comparison of inherent mutagenic activity of thirty BaP derivatives in *Salmonella typhimurium* TA98 and in Chinese hamster V79 cells.. 6-153

Table Page

6-32 Mutagenicity of carcinogenic hydrocarbons to Chinese hamster V79 cells co-cultivated with Syrian golden hamster embryo cells...... 6-157
6-33 Induction of ouabain- and 8-azaguanine-resistant mutants by different chemical carcinogens.................................. 6-158
6-34 Induction of temperature-resistant mutants in Chinese hamster ovary temperature-senstitive cells by different chemical carcinogens... 6-159
6-35 Mutagenic response of the X chromosomes in *Drosophila*............. 6-167
6-36 Mutagenic response of the BB loci in *Drosophila*.................. 6-169
6-37 Agents producing early fetal deaths and/or preimplantation losses significantly beyond control limits but requiring further verification because of lack of internal consistency............ 6-171
6-38 Comparison of mutagenicity in Ames assay with carcinogenicity of polynuclear hydrocarbons.................................... 6-173
6-39 Induction of sister-chromatic exchanges by intraperitoneal injection of POM in Chinese hamsters........................... 6-176
6-40 Effect of 3-methylcholanthrene on pregnancy in mice............... 6-179
6-41 Data on mouse embryos treated with 0.01 ml of 3-methylcholanthrene. 6-180
6-42 Effect of benzo[a]pyrene administration to pregnant mice on pulmonary adenoma formation in the progeny...................... 6-183
6-43 Effect of benzo[a]pyrene administration to pregnant mice on skin papilloma formation in the progeny............................. 6-184
6-44 Summary of epidemiological evidence of carcinogenicity............ 6-189
6-45 Summary of observations on observed deaths and relative risks of death from all cancers, respiratory cancer, and cancer of the digestive tract... 6-192
6-46 Average annual age, race, and sex specific mortality rates per 100,000 population for cancer of the lung in the United States, 1948-49... 6-206
6-47 Age-adjusted cancer-incidence rates per 100,000 population, by primary site and sex for urban and rural areas.................. 6-207
6-48 Ratios of age-adjusted mortality rates, 1950 to 1969, among white males in petroleum industry counties to those in control counties by cancer site... 6-211
6-49 Ranking of the cities investigated by the values of the atmospheric air pollution indices and death rate in the population from cancer of the lungs and bronchi................................ 6-214
6-50 Polycyclics content (ppb) of dried microalgae as a function of geographical location.. 6-224
6-51 Effect of aromatic hydrocarbons in marine bacteria................ 6-227

1. EXECUTIVE SUMMARY

This document was prepared in response to the Clean Air Act Amendments of 1977 which add Section 122 to the 1970 Act requiring the Administrator to decide whether atmospheric emissions of polycyclic organic matter (POM) may reasonably be anticipated to endanger public health. This document reviews POM data on chemical and physical properties, atmospheric forms, atmospheric fate and transport, measurement techniques, ambient atmospheric levels, toxicology, and occupational health and epidemiology. Another document [Multimedia Health Assessment Document for Polycyclic Organic Matter, J. Environ. Pathol. Toxicol. (in press, 1980)] has been prepared for EPA by Syracuse Research Corporation and covers POM exposure from air, food, and water sources.

1.1 DEFINITION AND FORMATION

The two POM chemical groups most commonly found in ambient air are polycyclic aromatic hydrocarbons (PAH), such as the well known carcinogen benzo[a]pyrene (BaP) and the PAH nitrogen analogs. In addition, a small number of oxygen-containing POM have been detected in ambient air. The major environmental sources of POM appear to be the combustion or pyrolysis of materials containing carbon and hydrogen. Although hundreds of individual compounds could be formed under various combustion or pyrolysis conditions or from various carbon-emitting sources, only about 25 parent PAH and 32 nitrogen and oxygen analogs have been quantitatively analyzed so far in ambient air. The failure to detect POM may be attributable to the low concentrations of some compounds and a lack of an appropriate quantitative analytical method for these agents (the presence of over 100 individual PAH in ambient air has been documented, but quantitative data are not available).

It is generally agreed that POM compounds are associated with suspended particulate matter from both mobile and stationary sources, principally respirable particles. Many of the compounds are susceptible to oxidation or photochemical reactions in laboratory experiments, but this susceptibility varies considerably among individual compounds, depending upon the physical form of POM tested and the reaction conditions used. However, available monitoring data suggest that many POM compounds associated with particulate matter are probably stable in ambient air for several days. A recent study in Norway has demonstrated that at least 20 PAH associated with particulate matter are stable enough in the atmosphere to travel from England or France to Norway.

1.2 ANALYTICAL METHODS

A number of methods are available for sampling POM from various sources and for the analysis of collected samples for quantification of individual compounds. POM in ambient air are usually collected as particulate matter on filters. Some of the more volatile POM may not be efficiently collected, but the collection efficiency for POM (including BaP) has not been determined. Because of the large number of individual compounds in the POM family, careful fractionation and separation procedures are necessary to ensure accurate quantitation. Distinquishing between individual compounds is important because slight alterations in chemical structure modify carcinogenic activity, and thus alter the potential health hazard.

There are several relatively simple, inexpensive analytical procedures that are capable of reliably measuring several individual POM compounds. However, as the number of compounds to be measured increases, the cost and time of analysis increase dramatically. It is apparent that not all of the individual POM compounds can be monitored on a routine basis because of the cost and labor that would be required. However, the current practice of monitoring only BaP seems equally unacceptable. Since BaP levels are not necessarily representative of the carcinogenic activity of particulate fractions collected from ambient air, in the future a multi-component analysis of POM compounds would be more desirable. The actual components selected should be easy to determine analytically and should be representative of those POM air pollution fractions which demonstrate carcinogenic activity.

1.3 AMBIENT CONCENTRATIONS

While POM compounds have been detected in ambient air, the most extensive data available are on BaP. The following trends have been noted for BaP: the median urban value declined from 3.2 ng/m^3 in 1966 to 0.5 ng/m^3 in 1975, an 80 percent decrease; levels in the winter were higher than in the summer, probably due to greater coal consumption; the levels in urban cities with coke ovens were 40 to 70 percent higher than in cities without coke ovens, but this is thought to be due in part to higher heating and industrial emissions in those cities. Some correlation between the amount of BaP and the amount of other PAH has been noted, but there are several exceptions. These exceptions suggest that the relative amount of each individual POM compound depends upon local combustion or pyrolysis sources.

The accuracy of the absolute values of BaP is unknown because of the lack of information on the collection efficiencies, oxidation during collection, or degradation in the time between collection and analysis. However, the observed trend of overall decreasing BaP levels is believed to be essentially valid, even if absolute levels are difficult to determine. Nevertheless this trend cannot be used to imply that the concentration of other POM is declining or that the carcinogenic activity of POM fractions of air particulates is reduced. For the most part, monitoring data for the other POM compounds have been collected only once at a limited number of sites.

1.4 HEALTH AND ECOLOGICAL EFFECTS

The major human health concern over exposure to POM is the possible development of cancer. It is well established that POM-containing extracts of particulate air pollutants are carcinogenic when painted on the skin of rodents or injected into newborn mice. Although it cannot be unequivocally stated that any of the POM are human carcinogens, several of these compounds are among the more potent animal carcinogens known to exist.

POM gain ready access to the body's circulation either by inhalation, ingestion, or contact with the skin. In their parent form, however, POM produce no major adverse effects. Instead, these compounds must first be metabolized by enzyme systems of the body to produce chemically reactive intermediates which are then capable of inducing cancer. As would be expected, the alteration of enzymic activity involved in the formation of reactive POM metabolites can have a marked effect on the carcinogenic process. This enzyme activity can be either enhanced or depressed by exposure to noncarcinogenic chemicals (such as food additives and pollutants), drugs, and naturally occurring agents in the diet. Moreover, a wide variation exists in the drug-metabolizing enzyme activity of humans; and it may be that the degree of this activity is a determinant for the susceptibility of humans to carcinogenic POM. The body also possesses mechanisms to repair carcinogen-induced damage, and the individual capacity for such processes is another likely determinant of susceptibility.

Numerous agents have also been identified which either promote or inhibit the carcinogenicity of POM when simultaneous exposure occurs, and their mechanism of action is as yet undetermined. Because of the diversity of human lifestyles and the multitude of chemicals to which humans are exposed that may either enhance or diminish their susceptibility, human response to carcinogenic POM is expected to vary considerably. Moreover, the risk of carcinogenic POM exposure to individuals who are in danger of developing cancer from other causes (e.g., radiation, chemicals, viruses) has never been fully evaluated. It is for these reasons that animal studies may not accurately reflect the cancer risk to humans of POM exposure.

In studies with animals, an apparent "threshold" level for POM exposures is often seen, below which no carcinogenic response is produced. This may be due to the fact that body defense mechanisms are capable of deactivating certain minimum amounts of a carcinogenic substance. On the other hand, in experimental situations it is not surprising to observe deviations from the predicted number of responses at very low doses, simply because limited numbers of animals are generally employed. Furthermore, it has been shown in animal studies that, even at levels which do not produce tumors, BaP can still interact with critical cellular constituents (e.g., DNA) in a dose-related fashion. Such interaction is regarded to represent an initial event in malignant transformation. Therefore, no one can presently say whether there exists a safe level for human exposure to POM. However, in real-life situations most scientists agree that the effects of carcinogenic POM will most likely be a continuous function of dose, no matter how small.

Whereas exposure to POM in occupational situations can clearly be associated with increased lung cancer development, epidemiological evidence in community settings does not permit a definitive conclusion. This is primarily because of the overriding impact that cigarette smoking has on POM exposure compared to inhalation of POM-containing particulate air pollutants. Nevertheless, when smoking habits are taken into consideration, urban residents have a two-fold risk for the development of lung cancer in relation to residents in rural environments. Since no other variables (e.g., lifestyle, medical care, etc.) can adequately account for this difference, most investigators feel it is reasonable to attribute at least some of this lung cancer excess among urban dwellers to the higher relative concentration of POM in city air. Moreover, it has been shown that, in some cases, cities having the highest levels of POM-containing pollutants in the air also report a higher rate of lung cancer mortality than other cities. Even among migrant groups who leave these polluted environments, an excess of lung cancer still appears while they are residing in an adopted country. Thus, it is evident that lifetime exposures to POM may not be necessary to result in an increased cancer risk.

Due to the relationship between molecular events involved in carcinogenesis and mutagenesis, it is not surprising that numerous POM produce mutations in certain animal and bacterial cells in culture. However, POM have not been conclusively shown to produce mutations in either animals or man which can be passed from one generation to the next. Furthermore, there is no evidence to indicate that POM in the air may be producing mutations in lower organisms in the environment, or that POM are presently disturbing the ecological balance in any way. POM are apparently not bioaccumulated in any species, nor are they transferred through the food chain. On the other hand, the high sensitivity of certain fish and amphibians to carcinogenic chemicals suggests that specific adverse ecological effects may result from low levels of contamination.

In conclusion, it is evident that the risk of lung cancer development by exposure to POM is real. The magnitude of that risk to the general population, however, cannot be accurately determined based on our present knowledge. It is apparently much less than the lung cancer risk associated with cigarette smoking, since POM exposure from smoking is far greater than that from inhaling polluted air. The involvement of POM in producing cancers of other sites (e.g., stomach, bladder) is also a possibility which has not been thoroughly explored in human populations. Nevertheless, in conjunction with smoking and the other foreign chemicals (carcinogenic and noncarcinogenic) to which man is exposed, it is likely that the absolute risk of POM exposure at any concentration is magnified in real-life situations. Thus, in adopting the most conservative approach for evaluating the human health hazard of POM, most scientists conclude that although the risk of cancer by exposure to low doses of POM is probably very small, it will not be possible to completely eliminate the risk unless all exposures are prevented.

2. INTRODUCTION

A number of documents[1,2,3] have reviewed polycyclic organic matter (POM) in considerable detail, but a considerable amount of information has been developed since then which needs to be evaluated in determining whether the release of POM into the atmosphere will endanger public health. The Clean Air Act Amendments of 1977 amended Part A of Title I of the 1970 Act by adding Section 122 as a new section entitled, "Listing of Certain Unregulated Pollutants." The new section reads as follows:

> Not later than one year after date of enactment of this section (two years for radioactive pollutants) and after notice and opportunity for public hearing, the Administrator shall review all available relevant information and determine whether or not emissions of radioactive pollutants (including source material, special nuclear material, and by-product material), cadmium, arsenic and polycyclic organic matter into the ambient air will cause, or contribute to, air pollution which may reasonably be anticipated to endanger public health. If the Administrator makes an affirmative determination with respect to any such substance, he shall simultaneously with such determination include such substance in the list published under section 108(a) (1) or 112(b) (1) (A) (in the case of a substance which, in the judgment of the Administrator, causes, or contributes to, air pollution which may reasonably be anticipated to result in an increase in mortality or an increase in serious irreversible, or incapacitating reversible, illness), or shall include each category of stationary sources emitting each substance in significant amounts in the list published under section 111(b) (1) (A), or take any combination of such actions.

In an effort to comply with the legislated mandate, this publication has been prepared as part of the basis to allow the Administrator to decide whether or not POM emissions cause or contribute to air pollution which may reasonably be anticipated to endanger public health. Given that the above determination is positive, this document will assist the Administrator in determining which regulatory mechanism is the most appropriate for POM.

In order to accomplish this objective, this document has reviewed the following information on POM: chemical and physical properties, form in ambient air, formation, fate and transport, measurement techniques, ambient levels, toxicology, and occupational and epidemiological information. The status of control technology for POM has not been considered in this report.

The scientific literature has been reviewed through 1977 and the early part of 1978. Because POM includes literally hundreds of individual compounds, all the papers ever published relating to POM in the environment and their effects could not be cited. Emphasis was placed upon those chemicals and chemical groups which had the highest potential for affecting public health. For this reason two chemical groups -- the polycyclic aromatic hydrocarbons (PAH), including benzo[a]pyrene (BaP), and the aza arenes -- were focused upon because of their association with carcinogenesis.

2.1 REFERENCES

1. Particulate Polycyclic Organic Matter. National Academy of Sciences. Washington, D.C. 1972, 361 pp.

2. Scientific and Technical Assessment Report on Particulate Polycyclic Organic Matter (PPOM). U.S. Environmental Protection Agency. Washington, D.C. Publication No. EPA-600/6-75-001. 1975.

3. Preferred Standards Path Report for Polycyclic Organic Matter. U.S. Environmental Protection Agency. Office of Air Quality Planning and Standards. North Carolina. 1974.

3. PHYSICAL AND CHEMICAL DATA

3.1 DEFINITION AND FORMATION

Polycyclic organic matter (POM) can include many chemical groups as indicated by the following list developed by an EPA Task Force in 1975:[1]

Polycyclic aromatic hydrocarbons (PAH) (see Table 3-1)
Aza arenes (arenes containing a ring nitrogen) (see Table 3-2)
Imino arenes (ring nitrogen with a hydrogen) (see Table 3-2)
Carbonyl arenes (see Table 3-3)
Dicarbonyl arenes (quinones) (see Table 3-3)
Hydroxy carbonyl arenes
Oxa arenes and thia arenes
Polychloro compounds
Pesticides (e.g., aldrin, chlordane, DDT)

Chemically, any organic compound that contains two or more rings could be considered a POM. However, of major concern are the carcinogenic polycyclic aromatic hydrocarbons (PAH), such as benzo[a]pyrene, and their nitrogen analogs, aza and imino arenes, which are formed during organic combustion processes.[2,5,6] Because of their common sources, their existence in urban air, and the considerable experimental data on their carcinogenic effects, the PAH and the nitrogen analogs have received the most attention, and therefore this report has focused on these POM. However, some of the other arenes (for example, 7H-Benz(de)anthracen-7-one) have been detected in urban atmospheres.[3] The latter compounds will be considered in this report even though a limited amount of data is available.

POM may be formed in most combustion or elevated temperature processes which involve compounds containing carbon and hydrogen. The amounts and types of individual chemicals that are formed are dependent upon the starting hydrocarbons and the conditions of combustion or pyrolysis. Although the exact intermediates that are postulated vary considerably, free-radical paths are commonly suggested at the high temperatures attained in the flame front (500 to 800°C) or under pyrolysis conditions. Badger[4] first postulated the synthetic route to POM formation outlined in Figure 3-1. His pyrolysis studies were conducted by passing the hydrocarbon vapor in nitrogen through a silica tube at 700°C. Although the use of nitrogen atmospheres has been criticized as lacking relevance to actual combustion,[5] the reducing conditions are similar to those of the oxygen-deficient environments of a flame, and the data are in good qualitative agreement with the POM combustion products formed.[2] For example, Boubel and Ripperton[7] found that benzo[a]pyrene (BaP) is produced during combustion even at high percentages of excess air, but that the amount of BaP is greater at lower percentages of excess air. Lending support to the postulated route to POM, Badger and Spotswood[8] pyrolyzed toluene, ethylbenzene, propylbenzene, and butylbenzene and obtained the highest yields of benzo[a]pyrene with butylbenzene. Badger and Spotswood[9] also found that when 1,3-butadiene is pyrolyzed with pyrene at 700°C, no increase in the yields of benzpyrenes is observed, which suggests that Diels-Alder type reactions are probably not important.

More recent studies tend to confirm most of the mechanism proposed by Badger.[4] Crittenden and Long[10] determined the chemical species at various flame heights of oxy-acetylene and oxy-ethylene flames. Compounds identified

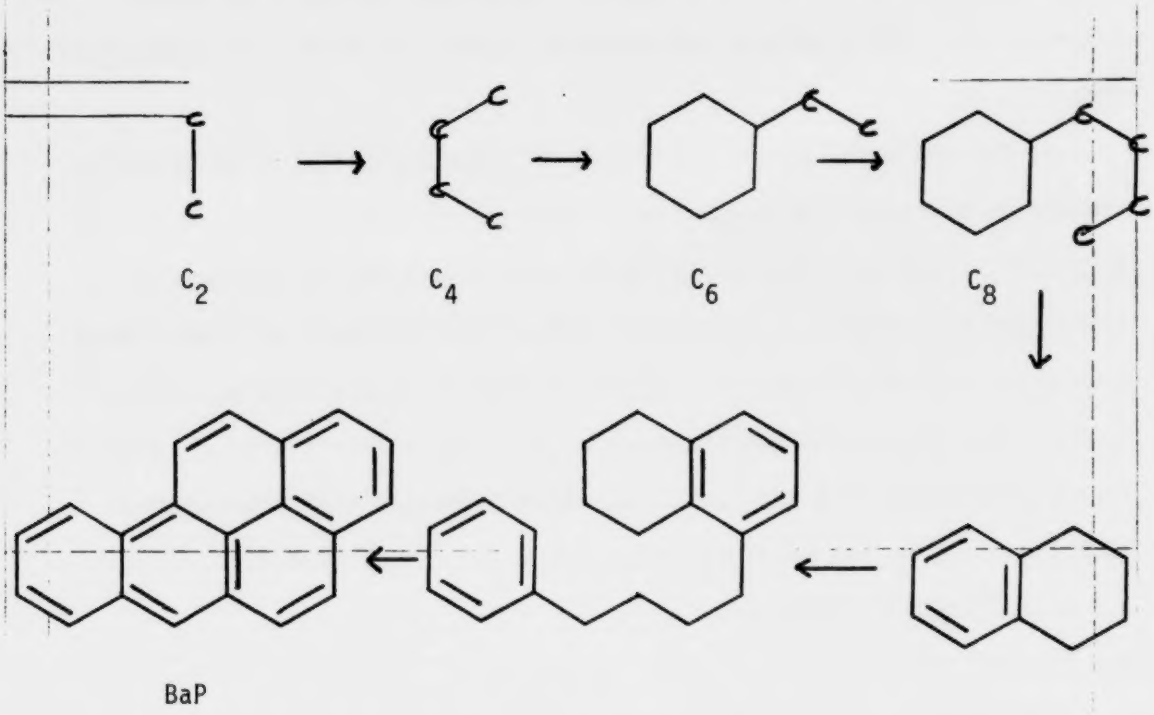

C
C
C
C
C
C
C
C
C
C
C
C
C
C
C
C
C
C
C
C
C_2
C_4
C_6
C_8
BaP

Figure 3-1. Mechanism of benzo[a]pyrene formation.[4]

suggest that the C_2 species react to form C_4, C_6, and C_8 species, and that reactions involving styrene and phenylacetylene are probably important in the formation of polycyclic aromatic hydrocarbons. Also, a $C_{10}H_{10}$ species was detected in the gases of both flames and, although its identity is uncertain, it may correspond to the C_6-C_4 species (butylbenzene, phenylbutadiene, or related radicals) postulated by Badger.[4]

With the Badger step-wise sequence mechanism, various free radical intermediates, and aliphatic and aromatic fuels, one could form hundreds of POM.

By far the most significant source of atmospheric POM is pyrolitic or combustion processes, although some investigators have suggested that some polycyclic aromatic hydrocarbons in the environment may be synthesized by plants and microorganisms[11] and that part of the PAH could be from natural combustion sources.[12] However, recent studies of aqueous sediment cores[13] suggest that the concentrations detected correspond relatively well with energy production from various fuels, which indicates that anthropogenic combustion may be the major source of PAH even in aqueous media.

3.2 STRUCTURE AND PROPERTIES

3.2.1 Chemical Structure

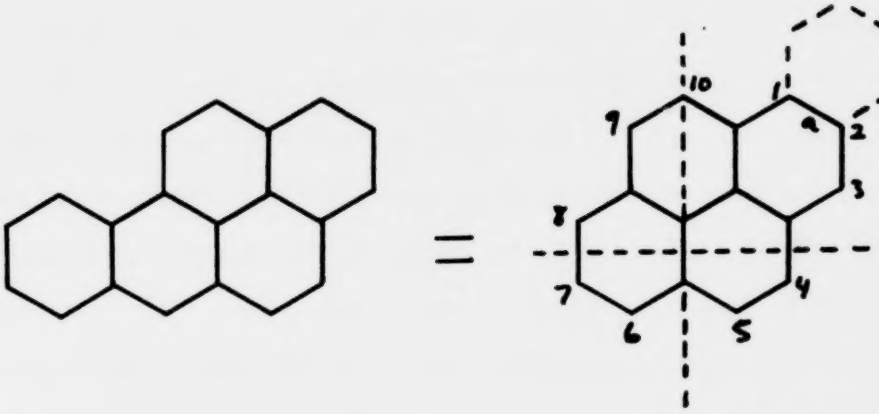
The nomenclature of POM compounds has suffered from considerable ambiguities in the past due to different peripheral numbering systems. For example, carcinogenic benzo[a]pyrene was named "3,4-benzpyrene" by American scientists and "1,2-benzpyrene" by European workers, while the noncarcinogenic isomer, benzo[e]pyrene, was named "1,2-benzpyrene" by American scientists and "4,5-benzpyrene" by European scientists. Thus, 1,2-benzpyrene could be the carcinogenic or noncarcinogenic isomer, depending upon where a scientist worked.[14]

The currently accepted nomenclature is that adopted by the International Union of Pure and Applied Chemistry (IUPAC) and by Chemical Abstracts Service.[2] Of major importance are the following rules that determine the orientation from which the numbering is assigned:[2,14-18]

1. The maximum number of rings lie in a horizontal row;
2. As many rings as possible are above and to the right of the horizontal row;
3. If more than one orientation meets these requirements, the one with the minimum number of rings at the lower left is chosen.

The carbons are then numbered in a clockwise fashion, starting with the first most counterclockwise carbon which is not part of another ring and is not engaged in a ring fusion. Letters are assigned in alphabetical order to faces of rings, beginning with "a" for the side between carbon atoms 1 and 2 and continuing clockwise around the molecule; ring faces common to two rings are not lettered. Thus, benzo[a]pyrene would have a benzene ring fused to the "a" bond of the parent pyrene structure (Figure 3-2).

The following tables provide lists of the POM that in most instances have been detected in air. However, some compounds (e.g., 7,12-dimethylbenz[a]anthracene and 3-methylcholanthrene) have been included in the table even though they are synthesized compounds which are not formed during combustion and are not present in ambient air. These synthetic compounds were included because they are used as model compounds in experimental carcinogenesis studies and have provided fundamental information concerning mechanisms of tumor formation. The chemical names, synonyms, structures, numbering


10
1
a
2
3
4
5
6
7
8
9

benzo[a]pyrene

pyrene

Figure 3-2. Accepted nomenclature for benzo[a]pyrene.

system, and acronyms used throughout the report, along with their relative carcinogenicity, are presented. The tables (3-1, 3-2, and 3-3) are divided into polycyclic aromatic hydrocarbons (PAH), aza and imino arenes, and miscellaneous other POM that have been detected in air. The structures are presented in the same way as those in the NAS report, Particulate Polycyclic Organic Matter,[2] in that aromatic rings are shown as plain hexagons and a methylene group as CH_2. The indications of carcinogenicity, taken from the NAS report, were obtained from several Public Health Service (PHS) surveys of compounds tested for carcinogenicity.[19-23] In some instances, indications of carcinogenicity were obtained from individual references; in those cases the references are provided. The indications of carcinogenicity which are a summary of all routes correspond to the following simple code:

-	not carcinogenic
±	uncertain or weakly carcinogenic
∓	carcinogenic
++, +++, ++++	strongly carcinogenic

For the most part this system of indicating carcinogenicity is very approximate. For more detailed treatment of the available data see Chapter 6. Additional compounds besides those listed in the NAS report were added in this report when they have been detected in air. Older nomenclature are included in parentheses, and names with an asterisk indicate disagreement with standard numbering.

Table 3-1. POLYCYCLIC AROMATIC HYDROCARBONS

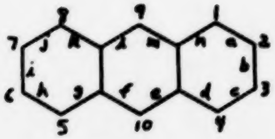
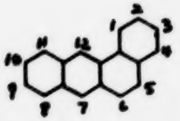
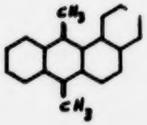
Compound	Anthracene	Benz[a]anthracene	7,12-Dimethylbenz[a]anthracene (9,10-dimethyl-1,2-benzanthracene)
Structure	 8 9 1 7 2 6 3 5 10 4 a b c d e f g h i j k l m n		 (not an air pollutant)
Formula	$C_{14}H_{10}$	$C_{18}H_{12}$	$C_{20}H_{16}$
Carcinogenicity	-	+	+ + + +
Acronym	A	BaA	DMBA
Physical properties			
Melting point, °C	216	159.5-160.5	122-123
Boiling point, °C	340	400	
Vapor pressure, Torr	1.95×10^{-4}	1.10×10^{-7}	
λmax, nm	308 323 338 355 375	314 327 341 359 376 386	296 345 364 384
Log ε	3.15 3.47 3.75 3.86 3.87	3.67 3.81 3.87 3.72 2.73 3.86	4.90 3.83 3.94 3.83
Solvent	EtOH-MeOH	EtOH	EtOH

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

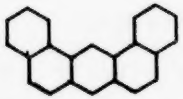
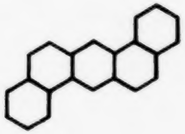
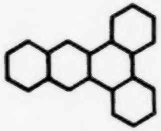
Compound	Dibenz[a,j]anthracene (1,2-6,7-dibenzanthracene)	Dibenz[a,h]anthracene (1,2-5,6-dibenzanthracene)	Dibenz[a,c]anthracene (1,2-3,4-dibenzanthracene)
Structure			
Formula	$C_{22}H_{14}$	$C_{22}H_{14}$	$C_{12}H_{14}$
Carcinogenicity	+	+ + +	+
Acronym		DBahA	
Physical properties Melting point, °C Boiling point, °C		266-266.5	
Vapor pressure, Torr			
λmax, nm Log ε Solvent		299 322 335 350 374 384 5.20 4.28 4.23 4.16 3.00 2.61 dioxane	

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

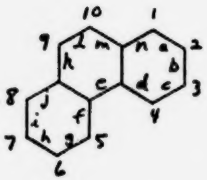
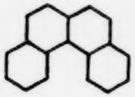
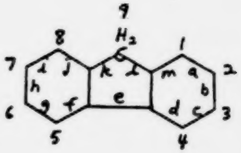
Compound	Phenanthrene	Benzo[c]phenanthrene (3,4-benzphenanthrene)	Fluorene
Structure	 10 1 2 3 4 5 6 7 8 9 a b c d e f g h i j k l m n		 9 H_2 1 2 3 4 5 6 7 8 a b c d e f g h i j k l m
Formula	$C_{14}H_{10}$	$C_{18}H_{12}$	$C_{13}H_{10}$
Carcinogenicity	-	+ + +	-
Acronym	PA	BcP	F
Physical properties			
Melting point, °C	101	68	116
Boiling point, °C	340		293
Vapor pressure, Torr	6.8×10^{-4}		
λmax, nm	309 314 323 330 337 345	296 303 315 325 353 372	
Log ε	2.40 2.48 2.54 2.52 3.40 3.46	4.13 4.07 4.06 3.67 2.63 2.38	
Solvent	EtOH-MeOH	EtOH	

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

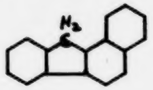
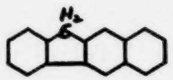
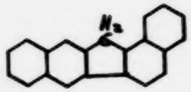
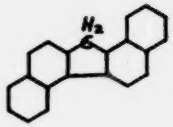
Compound	Benzo[a]fluorene (1,2-benzfluorene)	Benzo[b]fluorene (2,3-benzfluorene)	Dibenzo[a,h]fluorene (1,2-6,7-dibenzfluorene)	Dibenzo[a,g]fluorene (1,2-5,6-dibenzofluorene)
Structure				
Formula	$C_{17}H_{12}$	$C_{17}H_{12}$	$C_{21}H_{14}$	$C_{21}H_{14}$
Carcinogenicity	-	-	±	+
Acronym				
Physical properties				
Melting point, °C	189-190	208-209		
Boiling point, °C				
Vapor pressure, Torr				
λmax, nm				
Log ε				
Solvent				

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

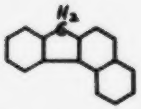
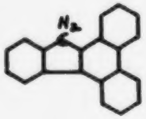
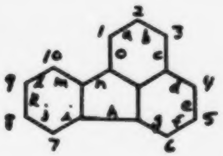
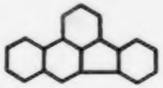
Compound	Benzo[c]fluorene (3,4-benzfluorene)	Dibenzo[a,c]fluorene (1,2-3,4-dibenzfluorene)	Fluoranthene	Benzo[b]fluoranthene (2,3-benzfluoranthene)
Structure			 1 2 3 4 5 6 7 8 9 10 a b c d e f g h i j k l m n o	
Formula	$C_{17}H_{12}$	$C_{21}H_{14}$	$C_{16}H_{10}$	$C_{20}H_{12}$
Carcinogenicity	-	±	-	+ +
Acronym			Flour	BbF
Physical properties				
Melting point, °C			110	167
Boiling point, °C			393	
Vapor pressure, Torr				
λmax, nm				
Log ε				
Solvent				

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

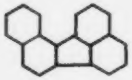
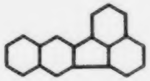
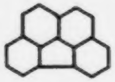
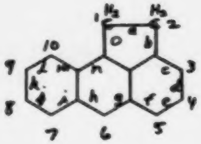
Compound	Benzo[j]fluoranthene (7,8-benzfluoranthene)	Benzo[k]fluoranthene (8,9-benzfluoranthene)	Benzo[ghi]fluoranthene	Aceanthrylene
Structure				 1 H2 H2 2 3 4 5 6 7 8 9 10 a b c d e f g h i j k l m n o
Formula	$C_{20}H_{12}$	$C_{20}H_{12}$	$C_{20}H_{10}$	$C_{16}H_{12}$
Carcinogenicity	+ +	-	-	-
Acronym	BjF	BkF		
Physical properties				
Melting point, °C	165-167	215-216		113
Boiling point, °C				
Vapor pressure, Torr		9.59×10^{-11}		
λmax, nm				
Log ε				
Solvent				

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

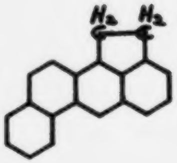
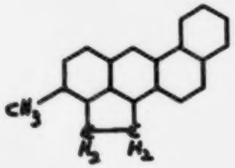
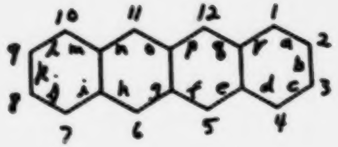
Compound	Benzo[j]aceanthrylene (cholanthrene)	3-Methylcholanthrene	Naphthacene (benzo[b]anthracene)
Structure		 (not an air pollutant)	
Formula	$C_{20}H_{14}$	$C_{21}H_{16}$	$C_{18}H_{12}$
Carcinogenicity	+ +	+ + + +	-
Acronym		MCA	
Physical properties			
Melting point, °C		179	341
Boiling point, °C			sublimes
Vapor pressure, Torr			
λmax, nm		no absorption above 300 nm	
Log ε			
Solvent			

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

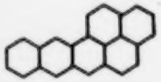
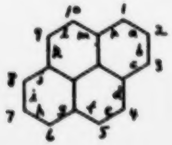
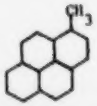
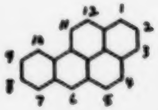
Compound	Naphtho[2,1,8-qra]naphthacene (naphtho[2,3-a]pyrene) (2,3-naphtho-1,2-pyrene)	Pyrene	1-Methylpyrene	Benzo[a]pyrene (1,2-benzpyrene) (3,4-benzpyrene*)
Structure		 10 1 2 3 4 5 6 7 8 9 a b c d e f g h i j k l m		
Formula	$C_{24}H_{14}$	$C_{16}H_{10}$	$C_{17}H_{12}$	$C_{20}H_{12}$
Carcinogenicity	-	-		+ + +
Acronym		P		BaP
Physical properties				
Melting point, °C		15		178.8-179.3
Boiling point, °C		>360		
Vapor pressure, Torr		6.85×10^{-7}		5.49×10^{-9}
λmax, nm				296 330 347 364 384 403
Log ε				4.76 3.76 4.12 4.38 4.48 3.60
Solvent				EtOH

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

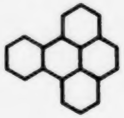
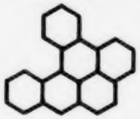
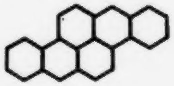
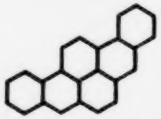
Compound	Benzo[e]pyrene (4,5-benzpyrene) (1,2-benzpyrene*)	Dibenzo[a,l]pyrene (2,3-4,5-dibenzpyrene) (Dibenzo[def,p]chrysene) (1,2-3,4-dibenzpyrene*)	Dibenzo[a,h]pyrene (1,2-6,7-dibenzpyrene) (Dibenzo[b,def]chrysene) (3,4-8,9-dibenzpyrene*)	Dibenzo[a,i]pyrene (2,3-6,7-dibenzpyrene) (4,5-8,9-dibenzpyrene*)
Structure				
Formula	$C_{20}H_{12}$	$C_{24}H_{14}$	$C_{22}H_{14}$	$C_{22}H_{14}$
Carcinogenicity	-	±	+ + +	+ + +
Acronym	BeP		DBahP	DBaiP
Physical properties				
Melting point, °C	178.9		320-320.5	281-282.5
Boiling point, °C				
Vapor pressure, Torr	5.54×10^{-9}			
λmax, nm			290 301 313 379 401 424 451	
Log ε			4.54 4.94 5.23 3.60 4.03 4.40 4.59	
Solvent			benzene	

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

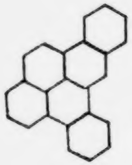
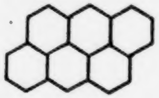
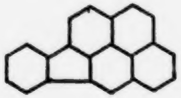
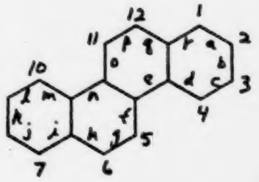
Compound	Dibenzo[a,e]pyrene	Dibenzo[cd,jk]pyrene (anthanthrene) (dibenzo[def,mno]chrysene)	Indeno[1,2,3-cd]pyrene (*o*-phenylenepyrene)	Chrysene
Structure				 1 2 3 4 5 6 7 10 11 12 a b c d e f g h i j k l m n o p q r
Formula	$C_{22}H_{14}$	$C_{22}H_{12}$	$C_{22}H_{12}$	$C_{18}H_{12}$
Carcinogenicity	+ + 24	-	+	$+^{25}$
Acronym		ANT	IP	CH
Physical properties				
Melting point, °C		257		254
Boiling point, °C				448
Vapor pressure, Torr				
λmax, nm				344 351 360
Log ε				2.88 2.62 3.00
Solvent				EtOH

Table 3-1 (Cont'd). POLYCYCLIC AROMATIC HYDROCARBONS

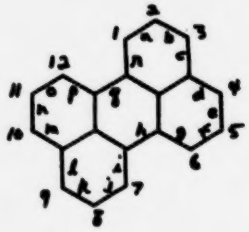
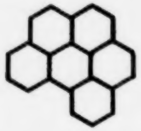
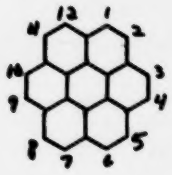
Compound	Perylene	Benzo[ghi]perylene	Coronene
Structure	 1 2 3 4 5 6 7 8 9 10 11 12 a b c d e f g h i j k l m n		 1 2 3 4 5 6 7 8 9 10 11 12
Formula	$C_{20}H_{12}$	$C_{22}H_{12}$	$C_{24}H_{12}$
Carcinogenicity	-	-	-
Acronym	Per	BghiP	Cor
Physical properties Melting point, °C Boiling point, °C	 273 ca 500	 273	 438 525
Vapor pressure, Torr		1.01×10^{-10}	1.47×10^{-12}
λmax, nm Log ε Solvent			

Table 3-2. AZA AND IMINO ARENES

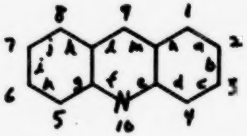
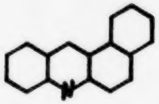
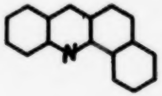
Compound	Acridine	Benz[a]acridine (1,2-benzacridine)	Benz[c]acridine (3,4-benzacridine)
Structure			
Formula	$C_{13}H_9N$	$C_{17}H_{11}N$	$C_{17}H_{11}N$
Carcinogenicity	-		
Acronym			
Physical properties			
Melting point, °C	111		
Boiling point, °C	>360		
Vapor pressure, Torr			
λmax, nm			
Log ε			
Solvent			

Table 3-2 (Cont'd). AZA AND IMINO ARENES

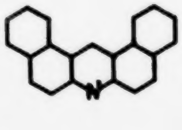
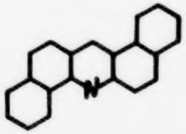
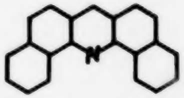
Compound	Dibenz[a,j]acridine (1,2-7,8-dibenzacridine)	Dibenz[a,h]acridine (1,2-5,6-dibenzacridine)	Dibenz[c,h]acridine (3,4-5,6-dibenzacridine)
Structure			
Formula	$C_{21}H_{13}N$	$C_{21}H_{13}N$	$C_{21}H_{13}N$
Carcinogenicity	+ +	++	±
Acronym			
Physical properties			
Melting point, °C	220	228	
Boiling point, °C			
Vapor pressure, Torr			
λmax, nm			
Log ε			
Solvent			

Table 3-2 (Cont'd). AZA AND IMINO ARENES

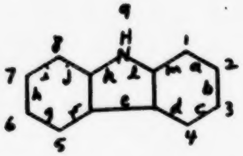
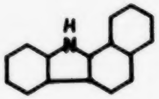
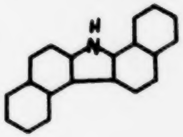
Compound	Carbazole	Benzo[a]carbazole (1,2-benzcarbazole)	Dibenzo[a,g]carbazole (1,2-5,6-dibenzcarbazole)
Structure	 9 H N 8 7 6 5 4 3 2 1 i j h g f e k l m a b c d		
Formula	$C_{12}H_9N$	$C_{16}H_{11}N$	$C_{20}H_{13}N$
Carcinogenicity	-	±	±
Acronym			
Physical properties			
Melting point, °C	246		
Boiling point, °C	355		
Vapor pressure, Torr			
λmax, nm			
Log ε			
Solvent			

Table 3-2 (Cont'd). AZA AND IMINO ARENES

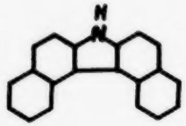
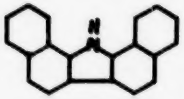
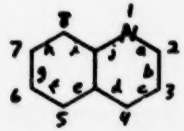
Compound	Dibenzo[c,g]carbazole (3,4-5,6-dibenzcarbazole)	Dibenzo[a,i]carbazole (1,2-7,8-dibenzcarbazole)	Quinoline
Structure			 1 N 2 3 4 5 6 7 8 a b c d e f g h i j
Formula	$C_{20}H_{13}N$	$C_{20}H_{13}N$	
Carcinogenicity	+ + +	+	+[53]
Acronym	DBcgC		
Physical properties			
Melting point, °C			
Boiling point, °C			
Vapor pressure, Torr			
λmax, nm			
Log ε			
Solvent			

Table 3-2 (Cont'd). AZA AND IMINO ARENES

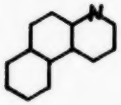
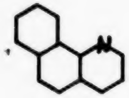
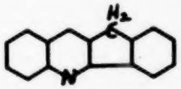
Compound	Benzo[f]quinoline	Benzo[h]quinoline	11 H-Indeno(1,2-b)quinoline
Structure			
Formula			
Carcinogenicity	-[54]		
Acronym			
Physical properties			
Melting point, °C			
Boiling point, °C			
Vapor pressure, Torr			
λmax, nm			
Log ε			
Solvent			

Table 3-2 (Cont'd). AZA AND IMINO ARENES

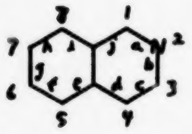
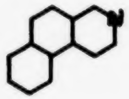
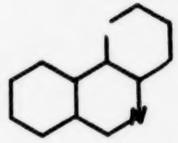
Compound	Isoquinoline	Benzo[f]isoquinoline	Phenanthridine
Structure	 8 1 7 N 2 6 3 5 4 h i j a b g f e d c		
Formula			
Carcinogenicity			
Acronym			
Physical properties			
Melting point, °C			
Boiling point, °C			
Vapor pressure, Torr			
λmax, nm			
Log ε			
Solvent			

Table 3-2 (Cont'd). AZA AND IMINO ARENES

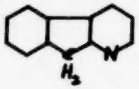
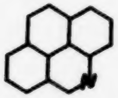
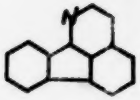
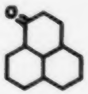
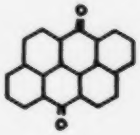
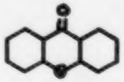
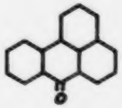
Compound	4-Azafluorene (5-H-indeno[1,2b]-pyridine)	4-Azapyrene (Benzo(lmn)phenanthridine)	1-Azafluoranthene (Indeno-[1,2,3-ij]isoquinoline)
Structure			
Formula			
Carcinogenicity			
Acronym			
Physical properties			
Melting point, °C			
Boiling point, °C			
Vapor pressure, Torr			
λmax, nm			
Log ε			
Solvent			

Table 3-3. MISCELLANEOUS POLYCYCLIC ORGANIC MATTER

Compound	Phenalene-1-one	Anthanthrone	Xanthene-9-one	7-H-Benz(de)anthracene-7-one (benzanthrone)
Structure				
Formula				
Carcinogenicity				
Acronym				
Physical properties				
Melting point, °C				
Boiling point, °C				
Vapor pressure, Torr				
λmax, nm				
Log ε				
Solvent				

3.2.2 Physical Properties

Most of the POM compounds are high melting/high boiling point solids that are extremely insoluble in water.[55] With the exception of the basic nitrogen heterocyclics (e.g., quinolines, acridines), the major POM compounds do not dissociate and, therefore, are found in the neutral fractions of extracts of particulate material.[5,6]

Only limited physical data are available on the numerous POM compounds. The information that is available is tabulated in Tables 3-1, 3-2, and 3-3. Melting points are available for most compounds and most values are considerably over 100°C (phenanthrene [101°C] and benzo[c]phenanthrene [68°C] are unusual exceptions).

Vapor pressures of the pure chemical are available for nine PAH. Depending upon the ring size and molecular weight of the compounds, the vapor pressure at 25°C of the pure compound varies from 6.8×10^{-4} Torr for phenanthrene (3 rings and 14 carbons) to 1.5×10^{-12} Torr for coronene (7 rings and 24 carbons).[24-26] This property has considerable impact on the amount of PAH that remain adsorbed on particulate matter in the atmosphere and retained on particulate matter during collection of air samples on glass fiber filters. In one study the following compounds experience the indicated losses at the various times when filtered air is drawn over particulate matter at 1.2 cubic meters per minute:[1,27] (1) tetracyclic or larger PAH, 2 hours - no loss; (2) tetracyclic PAH, 24 hours - some loss; and (3) pentacyclic or larger PAH, 3 weeks - no loss. The actual retention of POM on particulate during collection is likely to depend upon temperature, face velocity of the air with respect to the filter, and the adsorption characteristics of the individual POM.

The ultraviolet absorption spectra are also available for many compounds (Table 3-1). Most of the PAH absorbed light strongly at wavelengths longer than 300 nm.[28] The absorption spectra for benzo[a]pyrene (Figure 3-3) are typical. Because these compounds absorb light at wavelengths found in sunlight (>300 nm), it is possible that they may photochemically react by direct excitation (see Section 3.3.1). However, the available spectra were taken for PAH in organic solvents, while PAH in the environment are usually found associated with particulate matter and adsorbed PAH may have considerably different absorption properties.

3.2.3 Atmospheric Forms of POM

Relatively little information is available on the exact form of the various POM in the atmosphere. Because of the high melting points and low vapor pressures of most POM, the compounds are generally considered to be associated with particulate matter, either as pure material or adsorbed to other particulate matter.[2]

Some information is available on the relationship of POM to particle size, and this has particular significance with respect to respiratory tract entry, retention, and deposition. The aerodynamic size of particles will determine how much and where the particles are deposited. Relatively large particles (> diameter of 10 to 15 µm) are deposited in the nasal and oral cavities. Smaller particles will pass into the tracheo-bronchial regions. The actual deposition efficiencies within the respiratory tract will vary considerably. They are dependent upon the dimensions and configurations of the

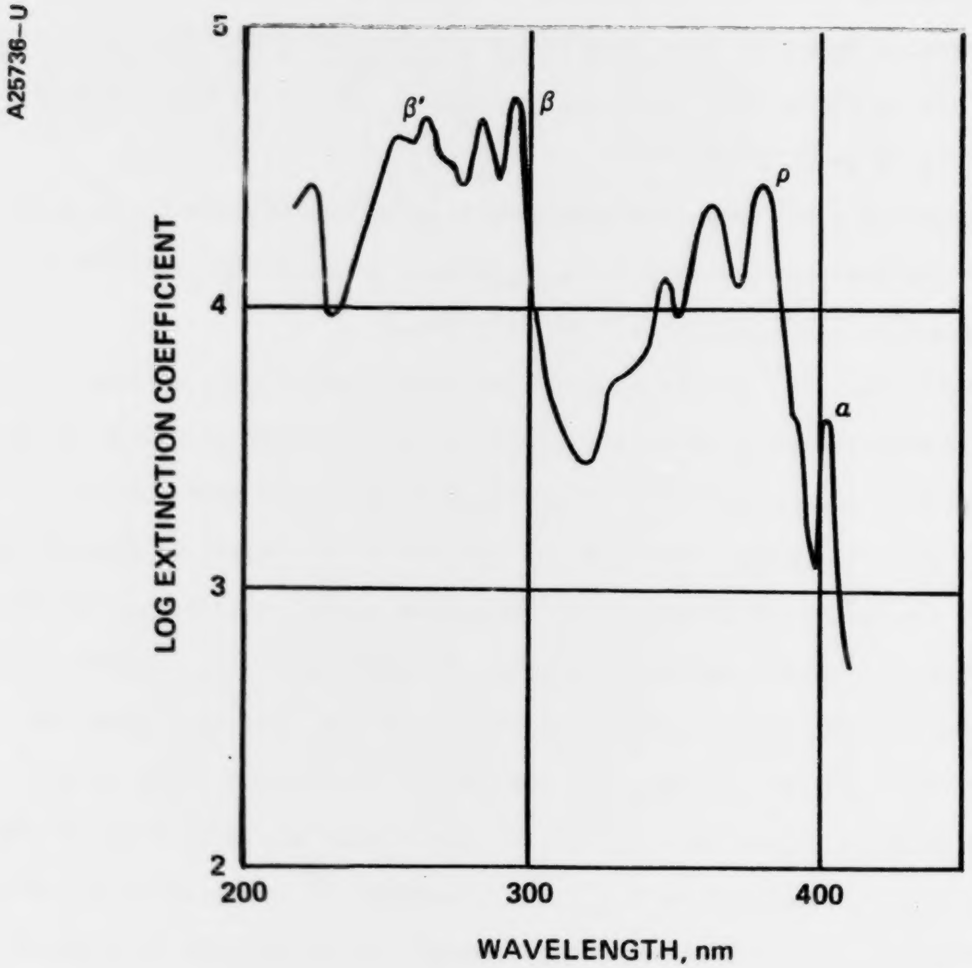


Figure 3-3. Absorption spectrum of benzo[a]pyrene in ethanol:[28] α, 4030 (3.60); ρ, 3845 (4.44), 3635 (4.36), 3470 (4.10), 3300 (3.76); β, 2965 (4.76), 2843 (4.66), 2740 (4.50); β', 2655 (4.66), 2540 (4.60); β", 2250 (4.44).

individual air path, the pattern and depth of respiration, and the characteristics of the particles.[29]

The particle size also affects the residence time of particulate matter. Urban aerosols appear to have a residence time without precipitation of 4 to 40 days for particles less than 1 μm in diameter and 0.4 to 4 days for particles 1 to 10 μm in diameter.[30]

Tebbens et al.[31] have separated particulates into different size fractions before extraction. They found a constant weight of BaP per unit weight of carbonaceous particles for all particle sizes.

DeMaio and Corn[32] collected particles with a two-stage elutriator. The first stage collected 50 percent of particles with a diameter of 4.6 μm and 100 percent of 6.5 μm particles. The second stage, which consisted of a glass fiber filter paper, collected 100 percent of all particles greater than 0.3 μm. The amount collected in the two stages varied considerably for the six compounds analyzed and depended upon the sampling period. Only 5 percent of the BaP was detected in stage one, while more than half the pyrene was found in stage one. Kertész-Sáringer and coworkers[33] used a four-stage Casella cascade impactor backed up with a glass fiber filter and determined the BaP on various size fractions at various times (September 11 to September 24; October 15 to November 14; and November 21 to December 16) of the year in Budapest. Their results (Figure 3-4) agree with those of DeMaio and Corn[32] in indicating that the great majority of BaP is in the respirable size range. In winter, when the concentration of BaP is the highest, the pollutant is associated more with smaller particles. Also, about half the weight of atmospheric BaP is associated with particles of radii less than 0.15 μm.

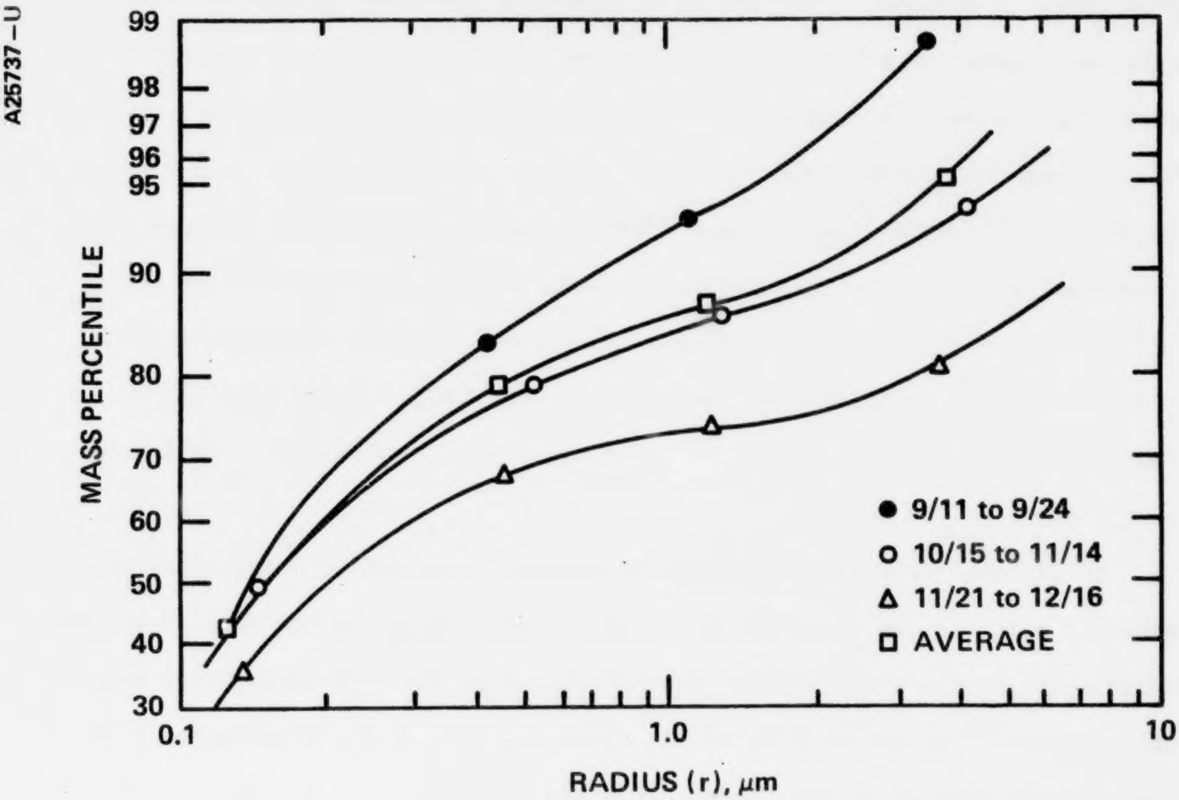


Figure 3-4. Size distribution of particles containing benzo[a]pyrene.[33]

Katz and Pierce[34,35] have conducted a detailed study of the relationship of PAH to relative size of particulate matter in Toronto, Ontario. Particulates were collected at five locations by a five-stage Anderson Hi-Vol cascade impactor. The size ranges (μm) for the five stages were: 1 ≥ 7.0; 2 = 3.3 to 7.0; 3 = 2.0 to 3.3; 4 = 1.1 to 2.0; and 5 ≤ 1.1. Twenty PAH compounds were monitored, but since the data for the other compounds were similar to data for BaP (Figure 3-5), only a few data on other isomers were presented. A seasonal relationship to submicron BaP particulates was found, with the highest mass fractions of submicron BaP particles (∿ 60 percent total aerosol mass) found during winter. At two locations, 40 to 60 percent of the PAH was associated with submicron particles; at another location only 6 to 14 percent PAH was thus associated. Differences were also found between the fractional mass of submicron BaP particulates samples taken at 50 feet and at 300 feet above the ground at different locations. Similar size distribution curves were also found for two oxygen heterocyclic PAH. These results demonstrate that BaP and PAH are associated primarily with small, respirable particles.

In elevated-temperature combustion systems, substantial amounts of POM probably exist in the vapor state. However, they appear to condense or adsorb on other particles rapidly once they are away from the high-temperature areas. Thomas et al.[36] determined the BaP in deposited soot in the upper portion of the glass chimney of a combustion system and compared it to the BaP in soot collected on a filter. No difference was detected, suggesting that BaP rapidly adsorbs onto particulate matter as soon as it leaves the combustion area. Thomas et al.[35] also determined the amount of BaP from the combustion

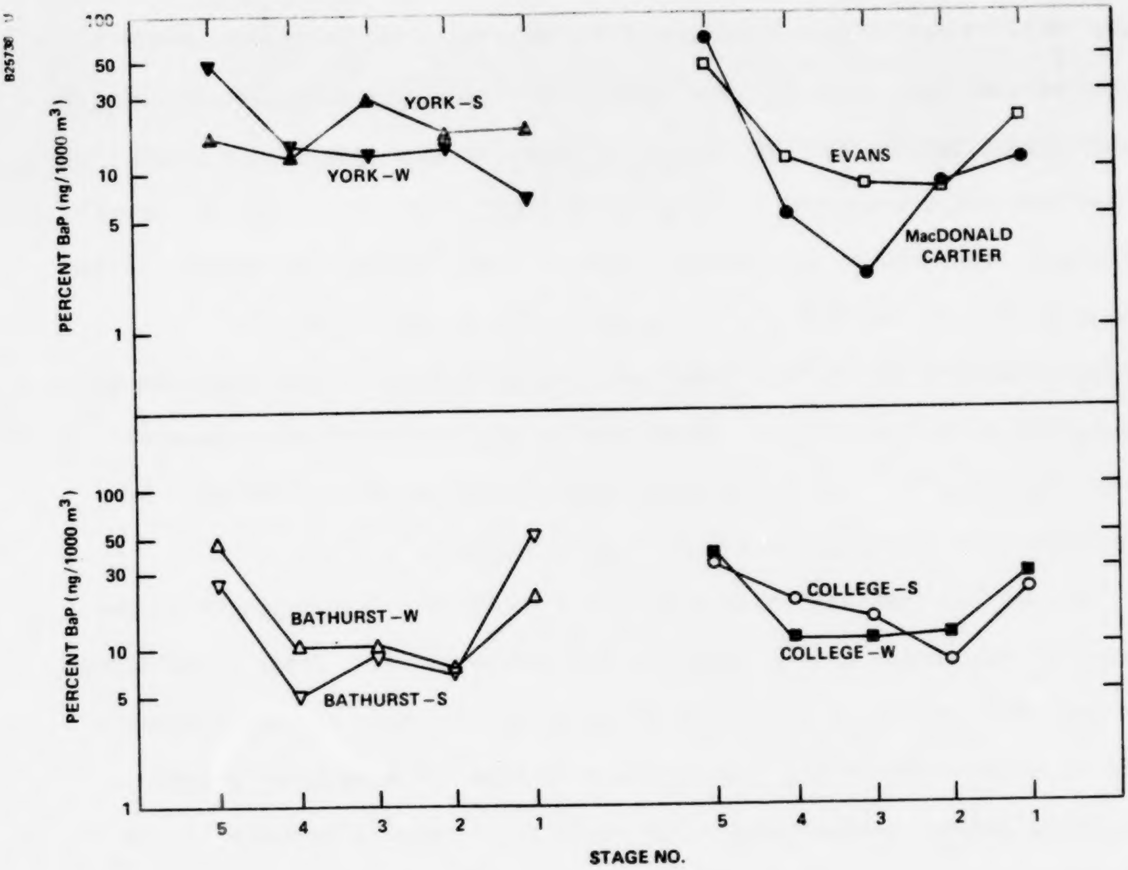


Figure 3-5. Percent mass of benzo[a]pyrene in relation to stage number for five sampling sites in Toronto, Canada.[35]

system collected on a filter (maintained at 80° to 90°C) or collected in a water and cold trap. Even at the elevated temperature of the filter, 96.3 percent of the total BaP was found on the filter. Sublimation of BaP off the particulate trap was ruled out.

Krstulovic et al.[37] have placed polyurethane plugs behind a standard glass fiber filter (1.0 μm retention with 98 percent efficiency) to collect particles that pass through. The authors refer to these particles as being in the gas phase because they act as gas or vapor in that they follow fluid streamlines and are subject to Brownian movement. Concentrations of several individual compounds in air samples taken in Rhode Island were greater on the foam plug than on the filter. For example, the concentration of dibenz[a,c]anthracene in Providence was 3709 ng/1000 m^3 on the foam plug but 806 ng/1000 m^3 on the filter. These results are consistent with those of Pierce and Katz,[34,35] which indicated that sizable amounts of PAH will be associated with particles less than 1 μm in diameter.

Natusch and Tomkins[38] have developed a mathematical description of the process of adsorption of PAH vapors on fly ash particles. Their calculations indicated that PAH would almost certainly exist as vapors at temperatures (>150°C) encountered in the stack systems of fossil-fueled power plants. However at ambient temperatures (-10° to 30°C), "essentially quantitative adsorption [to particulates] would be expected."[38] Their theoretical study also suggests that since "the extent of adsorption of PAH is proportional to the frequency of collision with the available surface area, then adsorption will result in small respirable particles being preferentially enriched (per unit mass) in potentially carcinogenic PAH."[38]

3.3 ATMOSPHERIC CHEMISTRY

The POM compounds emitted to the atmosphere have been shown to have considerably different stability. Unknown, however, is whether the degradation products are more or less toxic. Recent evidence by Pitts and coworkers suggests that in some instances (e.g., nitrogen dioxide reaction with benz[a]pyrene to form 6-nitrobenzo[a]pyrene and a mixture of 1-nitro and 3-nitrobenzo[a]pyrene) the products are more mutagenic.

The atmospheric stability of different POM compounds is dependent upon such factors as molecular structure, amount of available light, and presence of oxidizing pollutants.[1] Also, because POM in the atmosphere are mostly found in the atmosphere adsorbed to particulate matter, such factors as particle size (affecting rate of deposition and surface area exposed), porosity of the particle, and adsorption factors are also likely to be important to environmental stability. The rates that have been measured vary considerably depending upon the experimental conditions.

3.3.1 Photooxidation

Most investigators have used light sources that simulate sunlight (>300 nm). However, the form of the POM being photolyzed has been much more varied. Solutions of compounds are one of the most convenient forms for photolysis studies, but the relevance of solution photochemistry to photolysis of POM adsorbed on particulate matter is probably remote. Kuratsune and Hirohata[40] photolyzed 13 PAH in cyclohexane and dichloromethane with sunlight and with fluorescent lamps filtered through glass to provide wavelengths longer than 300 nm. They found that naphthacene was the most unstable of the PAH tested and that benzo[a]pyrene and alkyl derivatives of benz[a]anthracene were labile to light. The following compounds were stable under the experimental conditions: phenanthrene, fluorene, pyrene, fluoranthene, chrysene, benz[a]-anthracene, and benzo[k]fluoranthene. In another study by Kuratsune,[41] photolysis of BaP in benzene (light >280 nm and oxygen was present) allowed the isolation of 6,12-, 1,6-, and 3,6-benzo[a]pyrenequinones.

Because POM in the environment are usually found adsorbed to particulate matter, many investigators have studied the photolysis of POM naturally occurring on particulates from combustion, POM coated on some solid particles, and POM coated in thin films on a smooth surface. Typical of photolysis studies of pure POM adsorbed on solids is the work of Inscoe[42] who examined photodecomposition of PAH during thin layer chromatography. She exposed 15 PAH adsorbed on four different adsorbents (silica gel G, aluminum oxide G, cellulose powder, and acetylated cellulose) to ultraviolet light and ordinary room light. No changes were noted for phenanthrene, chrysene, triphenylene, and picene, but pronounced changes were detected for the other 11 compounds when silica gel and aluminum oxide were used; less dramatic changes were found with the other two adsorbents. Exposure to ordinary room light gave slower but similar results. The 11 compounds that were photosensitive were anthracene, naphthacene, benz[a]anthracene, dibenz[a,c]anthracene, dibenz[a,h]anthracene pyrene, benzo[a]pyrene, benzo[e]-pyrene, perylene, benzo[ghi]perylene, and coronene. Inscoe[42] also identified 1,6-pyrenedione and 1,8-pyrenedione as photoproducts of pyrene. As will be discussed later, some of these compounds appear to be fairly stable when adsorbed on natural particulate matter and, therefore, the above results may be only indicative of what occurs during thin layer chromatography.

Andelman and Suess[43] attempted to simulate photodecomposition of PAH in water by adsorbing BaP on calcium carbonate and irradiating aqueous suspensions with white fluorescent light. BaP was found to be unstable under laboratory illumination with 80 to 90 percent lost in 17 hours in 15 to 50 percent dioxane-water; but it is apparently somewhat dependent upon the liquid system since BaP is stable for 17 hours in 100 percent dioxane or 50 percent methanol/water.

A couple of investigators have examined the effect of some common air constituents on the photochemistry of adsorbed POM. Jager and Rakovic[44] adsorbed pyrene and BaP to fly ash and alumina from an acetone solution and irradiated the adsorbed compounds in a quartz tube filled with 10 percent SO_2 in air at room temperature and at 60° to 70°C. From irradiation of pyrene at room temperature, the authors were able to isolate many compounds containing sulfur, including 1-pyrenesulfonic acid (15 percent); from BaP, benzo[a]-pyrene-4-sulfonic acid was detected. Whether this type of reaction occurs in ambient air samples is unknown since the experimental concentration of SO_2 is much higher than is normally present in ambient air.

Geacintov[45] studied the photolysis of 20 PAH adsorbed on solid polystyrene fluffs in the presence of oxygen and nitric oxide. He found negligible photochemical degradation compared to quenching by the paramagnetic gases, O_2 and NO. However, the PAH were efficient photocatalysts for the formation of singlet oxygen, a reactive oxidizing agent which may react with the PAH.

Perhaps the earliest study of POM photochemistry was conducted by Falk and coworkers.[46] They noted that the amount of BaP in ambient air compared with other PAH was greater than one would expect on the basis of amounts emitted and thought this could be explained by a difference in atmospheric stability. They studied the stability of two forms of PAH: "pure" (dissolved in solvent and applied to a filter) and "adsorbed" (combustion particles collected on filter-sample divided into control and exposed sample). The samples were exposed to air and to a synthetic smog (1 hour of exposure equivalent to approximately 100 hours of exposure to natural smog) with and without irradiation. Their results are presented in Table 3-4. For most compounds, destruction of the "pure" samples is essentially similar in dark or light. However, BaP stability is significantly different with and without light. Also, the lower rate of reaction of the adsorbed form of BaP suggests that adsorption may provide some protection from photooxidative processes. The results for pure unadsorbed chrysene with light are difficult to explain. Falk et al.[47] concluded that the three-ring compounds disappear rapidly and that differences in atmospheric stability can explain why the ratio of pyrene to BaP is reversed following atmospheric residence. Also, adsorption on particulate matter seems to have a stabilizing effect compared to coating on a filter.

Tebbens et al.[31] also used the filter paper procedure of Falk et al.[45] to study photolysis of BaP by sunlight or simulated sunlight. With BaP deposited on various filters from an ether solution, different filter material resulted in different losses (60 to 92 percent loss). Tebbens et al.[31] also studied the chemical modification of BaP and perylene in smoke passed through

Table 3-4. PERCENT DESTRUCTION OF POLYCYCLIC AROMATIC HYDROCARBONS UNDER ATMOSPHERIC CONDITIONS[45]

	Pure Unadsorbed Compounds					Adsorbed on soot	
	Air in dark		Air in light		Smog in light	Air in light	Smog
	24 hr	48 hr	24 hr	48 hr	1 hr	48 hr	1 hr
Anthanthrene	44	49	42	44	--	5	55
Phenanthrene	39	61	34	60	--	-	--
Pyrene	24	43	20	42	83	1	58
Fluoranthene	17	20	16	24	59	4	59
Benzo[a]pyrene	0	0	21	22	50	10	18
Benzo[e]pyrene	--	--	--	--	--	7	51
Benzo[ghi]perylene	0	0	0	0	27	0	67
Coronene	0	0	0	0	5	--	--
Chrysene	0	0	11	0	15	--	--

a 22-foot dynamic flow chamber (Pyrex pipe surrounded with fluorescent lamps) by comparing the difference between irradiated and non-irradiated samples. They found that irradiation caused disappearance or modification of 35 to 65 percent of the original content and that oxygen, although its presence increases the rate of photodegradation, is not necessary for photoalterations.

Thomas et al.[36] used a dynamic flow-through chamber that was coupled to a furnace and controlled lighting having one-quarter the intensity of an average July noonday sun. They measured the BaP/soot at the entrance and exit of the chamber. A 58 percent decrease in the ratio was noted with the light turned on compared to when the light was off (retention time of 40 minutes). Thomas et al.[36] also examined the effect of particle size by coating glass beads (1 g of either 220 μm or 28 μm diameters) by evaporating the solvent. The beads were then exposed to sunlight in flasks and agitated hourly to expose new surfaces. BaP on small beads decreased 1 percent in 12 hours; on large beads, 1 percent in 7 days. The authors concluded that the 60 percent decrease in the 40 minutes retention time of the BaP/soot in the dynamic chamber may be related to the much greater surface area of the soot exposed to light.

Lane and Katz[48] have recently concluded that the previous experiments were not representative of actual atmospheric conditions, since the oxidant levels used were often too high and fluorescent lamps are poor simulators of sunlight. They used a dynamic flow reaction chamber (20-liter glass jar) with O_3 levels of zero to 2.28 ppm and irradiation from a Quartzline lamp. They studied the disappearance of 500 ng of BaP, benzo[b]fluoranthene (BbF), and benzo[k]fluoranthene (BkF), distributed in a thin dispersion in a petri dish.

The results presented in Table 3-5 show that BaP reacts rapidly with ozone compared to BbF and BkF, even in the absence of light. This is particularly significant because this type of nonphotochemical oxidation could occur during the normal 24-hour high-volume procedure used for sampling ambient air. The authors also found that the rate of decomposition slowed in time, suggesting that the surface-exposed layer reacted quickly but hindered the decomposition of subsurface material. However, it should be kept in mind that these results are based upon PAH coated in a thin film on glass. Whether these are the same results that would be obtained with PAH adsorbed on soot is unknown. Falk et al.[46] have shown that soot adsorption can have a stabilizing effect on BaP.

Relatively few studies have attempted to identify the photooxidative products. As indicated earlier, Masuda and Kuratsune[41] and Inscoe[42] have identified quinones of BaP and pyrene. This is consistent with the monitoring results of Pierce and Katz,[49] who tentatively identified five polycyclic quinones in air samples in Toronto. The seasonal increase of polycyclic quinones during the summer is compatible with a photochemical mechanism.

3.3.2 Oxidation and Nitration

As indicated in some studies in the previous section, light is not necessarily required for the atmospheric decomposition of POM. Falk et al.[46] found that for many PAH (excluding BaP) the amount of loss of an individual compound coated on a filter was the same in air in dark as in light. Also, the amount of loss of PAH adsorbed on soot was very high in a synthetic smog that had a high oxidant concentration. In addition, Lane and Katz[48] found that BaP reacted rapidly in reasonably low ozone concentrations

Table 3-5. THE HALF LIVES OF BaP, BbF, and BkF UNDER VARIOUS REACTION CONDITIONS[48]

Reaction conditions		Half lives, hr[a]		
Irradiation	Ozone concentration (ppm)	BaP	BbF	BkF
None	0.19	0.62	52.7	34.9
	0.70	0.4	10.8	13.8
	2.28	0.3	2.9	3.3
Quartzline Q500T/CL lamp	0.0	5.3	8.7	14.1
	0.19	0.58	4.2	3.9
	0.70	0.2	3.6	3.1
	2.28	0.08	1.9	0.9

[a] The half life was determined from the linear regression analysis of the initial linear portion of each decomposition curve.

(0.19 to 0.70 ppm) without irradiation and that the addition of irradiation had little effect on the rate. However, the other two PAH (BbF and BkF) were not nearly as susceptible to ozone oxidation. Thus, it appears that POM will be susceptible to air oxidation without light irradiation and that the reactivity is likely to vary for different structures.

Pitts et al.[39] have demonstrated that BaP deposited on glass fiber filters will react with O_3 and PAN to form oxygenated products and with NO_2 to form nitro derivatives when these gases are drawn through the filters. Exposure of BaP to 1 ppm NO_2 containing traces of nitric acid results in formation of compounds that were assigned the structure 6-nitro-, 1-nitro-, and 3-nitro-BaP (the latter two isomers were unresolved by TLC). Both the 6-nitro-BaP and the mixture of 1-nitro-acid 3-nitro-BaP were direct acting frameshift mutagens in the Ames assay. With BaP exposed to O_3, PAN, or smog, oxidation products including hydroxy-, dihydroxy- and dihydrodiol derivatives were detected. Perylene under similar experimental conditions (1 ppm NO_2) also formed a nitro derivative, 3-nitroperylene, which was a directly active mutagen in the Ames reversion assay even though the parent compound, perylene, shows no mutagenic activity with or without activation. All of these reactions were carried out on PAH deposited on glass-fiber filters. Whether these compounds would react similarly in the form found in the atmosphere (adsorbed to particulate matter) is unknown.

Commins[27] also provided suggestive evidence of nonphotochemical decomposition of PAH by collecting PAH particulate matter from air and comparing the results of immediate analysis to results from analysis after 1 year of storage in a sealed envelope (Table 3-6). Significant loss of lower molecular

Table 3-6. COMPARISON OF PAH CONTENT OF AIR SOLIDS BEFORE AND AFTER STORAGE[27]

PAH	Vapor pressure of pure compound[25,26]	Concentration of PAH, μg/g smoke		
		Analyzed immediately after collection	Analyzed 1 yr after collection	Loss, %
Fluoranthrene		225	18	92
Pyrene	6.83×10^{-7}	328	38	88
Benzo[a]pyrene	5.49×10^{-9}	111	76	32
Benzo[e]pyrene	5.54×10^{-9}	71	55	23
Anthanthrene		70	55	21
Benzo[ghi]perylene	1.01×10^{-10}	252	226	10
Coronene	1.47×10^{-12}	142	140	1

weight PAH was noted but little loss with the higher molecular weight compounds was found. This loss could be explained by evaporation of the lower molecular weight compounds rather than by oxidation.

3.3.3 Atmospheric Stability

The above results would suggest that many POM oxidize or photodegrade at a significant rate under atmospheric conditions. However, no satisfactory quantitative rate data are available. In contrast to the results reported above, Lunde and Bjørseth[50] published suggestive evidence that many POM are stable enough in the atmosphere to travel long distances. They monitored air samples in Norway which had different trajectories. They identified 20 different PAH and determined that samples with trajectories from western Europe contained about 20 times more PAH than samples with trajectories from northern Norway or stationary air from southern Norway. The PAH found in high concentrations under appropriate wind trajectories were phenanthrene, anthracene, methylphenanthrene/-anthracene, fluoranthene, dihydrobenzo[a&b]fluorenes, pyrene, benzo[a]fluorene, benzo[b]fluorene, 1-methylpyrene, benzo[c]phenanthrene, benz[a]anthracene, chrysene/triphenylene, benzo[b&k]fluorathene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-cd]pyrene, benzo[ghi]-perylene, anthanthrene, coronene. These results suggest that the above compounds are relatively stable in the atmosphere in the form in which they are naturally present.

3.4 SUMMARY AND CONCLUSIONS

The POM chemical groups most commonly found in ambient air are polycyclic aromatic hydrocarbons (PAH), such as benzo[a]pyrene, and their nitrogen analogs, aza- and imino-arenes. Other related compounds such as carbonyl arenes and dicarbonyl arenes (quinones) are less commonly detected.

The major environmental sources of POM appear to be from combustion or pyrolysis processes which use materials containing carbon and hydrogen. Current theory suggests a free-radical, step-wise, sequence mechanism which with various fuels could result in the formation of hundreds of POM.

Most of the POM are high melting/high boiling point solids that are very insoluble in water. At 25°C, the available vapor pressures of the pure compounds vary for the individual compounds from 10^{-4} Torr (3 rings) to 10^{-12} Torr (7 rings). The PAH are strong adsorbers of ultraviolet light of wavelengths from 200 nm to 400 nm.

It is generally agreed that most POM compounds are associated with suspended particle matter, with a large portion found with particles smaller than 1 μm. However, two- or three-ring compounds may be found partially in the vapor phase. Whether POM are condensed into discrete particles after cooling or are adsorbed on surfaces of existing particles is still unknown. Urban aerosols appear to have a residence time without precipitation of 4 to 40 days for particles less than 1 μm in diameter and 0.4 to 4 days for particles 1 to 10 μm in diameter. Thus, the atmospheric lifetime of a POM particle will be closely related to its particle size.

Experimental chemical and photochemical reactivities of POM on particulate matter vary considerably from half-lives of less than a day to several days. Particularly interesting are the different reactivities noted for the various compounds studied and the indication that reaction rates of compounds on smaller particle sizes are likely to be faster than those of larger particles. Light irradiation considerably accelerates the decomposition of some compounds but not others. Also, various experimental conditions have given contradictory results. One report has indicated that oxidation by ozone is the most important decomposition process for BaP in air, but many other reports have documented the photochemical susceptibility of BaP. However, monitoring data suggest that at least 20 PAH are stable enough in the form found in the atmosphere that they can travel long distances with particulate matter. Thus, although an exact residue time of POM is difficult to estimate from the available data, many of the POM compounds are stable enough to remain at significant concentrations during transport from the source to where they are inhaled. Although the smaller particles will have a longer atmospheric residence time, they will probably also react faster because of the greater surface area exposed to oxidants and light. All the available information does support previous conclusions that at least the outer layer of some POM adsorbed on particles is relatively reactive. However, this may mean that there is an inner layer of POM that would be relatively stable and perhaps available for elution by biological fluids following inhalation. The relatively long stability of several PAH based upon monitoring data is consistent with this possibility. Only limited information is available on the products of decomposition or their toxicologic properties. Quinones appear to be common atmospheric degradation products of PAH.

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4. SAMPLING AND ANALYTICAL METHODS FOR POM

4.1 INTRODUCTION

In vivo bioassays have demonstrated that the greatest carcinogenic activity of organic pollutants in air is associated (1) with the neutral fraction containing both polynuclear aromatic compounds (PAH) and their neutral nitrogen analogs, the aza-arenes, and (2) with the basic fraction containing the basic aza- and imino-arenes. Therefore, it is necessary to monitor these three classes of compounds in environmental samples both for assessment of carcinogenic potential and enforcement of any pollution discharge standard that may be set to control their emission. Excluding the natural sources, some of the contributing sources of POM in the air are from mobile sources entering the atmosphere as vehicular and aircraft emissions and from stationary sources that include home heating, power plants utilizing fossil fuels, refuse burning, road abrasions, and industrial processes. The monitoring of these POM compounds can be divided into four distinct steps: (1) sample collection; (2) desorption of the collected compounds from the collection media; (3) clean up and separation of the desorbed compounds; and (4) detection and analysis for quantitation.

The objective(s) of sampling must be defined clearly before the initiation of sample collection. Three primary objectives in the consideration of POM sampling include determination of (1) airborne concentrations of total suspended particulate matter, (2) distribution of particle size associated with the POM, and (3) chemical composition of the particles and their levels in the environment. In addition to these objectives, the selection of sampling site(s) and the implementation of appropriate sampling strategy play important roles in the acquisition of relevant data desired from ambient air sampling.

POM can be present in the air samples in three forms: (1) adsorbed on foreign particulate matter, (2) condensed POM in suspension with air, i.e., aerosol form, and (3) vapor phase. Evidently, POM from different sources will be present in all the three forms, although their proportion will depend on the nature of the source. Any collection method for monitoring POM should take these factors into consideration. The errors in most sample collection methods arise from the inability to collect either wholly or partly all three forms of POM, particularly the volatile components.

The desorption of the collected compounds is no less problematic. The particulate adsorbed part of the POM is bound firmly to the medium. This often leads to incomplete and unreproducible desorption. The selection of both the method of desorption and the nature of solvent (whenever used) play an important role in the recovery of POM obtained from this step.

The lack of clean up and separation of POM in environmental samples poses a serious hindrance to a better understanding of the composition. Yet separation of hard-to-separate isomers and the trace constituents is quite important. Small differences in the structure of these compounds may cause great differences in the biological activities. Also, due to the specificity of biological reactions, sometimes a trace component may produce more adverse biological effects than the predominant components. Therefore, the selection of the clean up and separation procedure will not only depend on the nature of the sample but also on the objective of the analysis.

With regard to the method of detection, any technique chosen must be sensitive and selective to provide interference-free quantitation of the trace components as well as the predominant ones.

Despite the greatest of precautions, there are some unavoidable losses, both during collection and analysis of POM. The losses during analysis alone may vary considerably depending on the nature of sample, method used, and the experience of the analyst. To secure quantitative data, internal standards must be used from the beginning of the analysis. This is usually done by spiking the sample with radioactive compounds such as C^{14}-benzo[a]pyrene, C^{14}-benzo[a]anthracene, or some other nonisotopic compounds not present in the original sample. A number of compounds including o-terphenyl, bibenzyl, and benzo[b]chrysene, have been used for the latter purpose.

The instability of the POM resulting from volatility, photosensitivity, and chemical transformation/degradation dictate that special precautions be used in handling these samples. Losses and chemical transformation during sampling and separation caused by these factors should be avoided whenever possible. The state-of-the-art of the four individual steps required for POM monitoring is discussed in the following sections with the above mentioned difficulties in mind.

4.2 SAMPLING

The POM sampling methods can be divided into four groups: (1) mobile sources, (2) tobacco smoke (3) stationary sources, and (4) ambient air.

4.2.1 Mobile Sources

The most important mobile source of POM in the air is vehicular exhaust. The sampling methods for POM from this source are treated in this section.

Certain vehicular parameters play important roles in the characteristics of POM emissions from this source. It is important to ascertain and define these parameters even before initiating any sampling procedures. Performance characteristics of the engine, its condition at the time of experimentation, and the fuel characteristics influence the nature and amount of POM emissions. The effects of performance characteristics, such as engine load, speed, cycle of operation, fuel-to-air ratio, and operating temperature, have been demonstrated by Spindt.[1] Gross[2] has shown the effect of accumulation of deposits in the combustion chamber of the engine on the POM emission. A number of investigators[3-7] have demonstrated the relationship between POM emission characteristics and fuel composition, namely, its aromaticity and PAH content.

A variety of procedures are available for sampling POM from automobile exhausts. It is not possible, however, to discuss all these procedures in detail. The general principle of sampling auto exhausts and references to a few procedures are presented here.

In one procedure,[8] the particulate matter and condensable vapors from auto exhausts are collected by a total condensation trap. In another procedure,[9] the exhaust aerosols from the automobile are passed through a glass cooler to bring the aerosol to an optimum temperature. The cooled aerosol is then passed through a fiberglass filter and finally through a double filter of silica gel. The POM collected at different stages of the system are individually eluted with organic solvents for their analysis.

Recently, Spindt[1] used an air dilution method for the collection of POM from auto exhausts. In this procedure, the hot exhaust aerosol is precooled to 75° to 170°C by a precooler and is mixed with clean and dry air. The air is chilled by passing it through a coil immersed in a bath of dry ice and methanol to a temperature that gives a diluted sample temperature of 20° to 30°C. The mixture is passed through a pretreated filtering system consisting of a fiberglass pad placed ahead of a glass fiber filter. The purpose of the fiberglass pad is to collect the bulk of the large soot particles before the aerosol comes in contact with the glass-fiber filter. The residual from the aerosol is finally passed through a Chromosorb-102 trap. The sample flow rate is measured by a dry gas test meter.

Irrespective of the collection method used, the efficiency of collection must be known and must be reproducible. This is hardly the case for samples collected from automobile exhausts. While some investigators[9] have described the efficiency of removal of particles of certain sizes, they did not report the overall efficiency. By the use of radioactive tracer, one investigator[1] reported the recovery efficiency of BaP from automobile exhausts as low as 6 percent, under no-load condition, but failed to determine whether the loss was principally during sample collection or the analytical procedure. It appears that there is a need for a standardized procedure for the collection of POM from automobile exhausts which can determine the collection efficiency and reproduce the technique with reasonable accuracy.

4.2.2 Tobacco Smoke

POM content in tobacco smoke depends primarily on the processing methods used for the manufacture of tobacco products, namely, the nature of the curing and drying process[10] and steps involving incorporation of additives such as flavoring materials. In addition, substantial amounts of POM originate from the paper used to roll the tobacco of the cigarette. In determining the POM content in tobacco smoke, it is important to realize that mainstream smoke and sidestream smoke do not necessarily have the same composition.[10] The sampling method, therefore, should specify the part of smoke that has actually been sampled.

For collection of mainstream smoke, the standard method consists of using a fully automatic smoking machine where the cigarettes are smoked to a butt length of 23 mm under standard conditions. These include a puff volume of 35 ml, puff duration of 2 seconds, and puff frequency of one per minute.[11] The collection of particulate matter is accomplished by an electrostatic precipitator.[10] In some methods, a cold-trap system replaces the electrostatic precipitator for the collection of smoke condensates.[12]

For collection of sidestream smoke, a wide glass tube fitted on the cigarette holder at the end opposite to the smoking side has been used.[10] The sidestream smoke from this tube is drawn through a side-arm and collected by means of two traps in series, packed with glass-wood and cooled to -70°C.

4.2.3 Stationary Sources

Emission from stationary sources can be divided into two groups: (1) stack emissions and (2) fugitive emissions from road-way dust.

4.2.3.1 Emissions from stacks--Several methods are available for collecting POM samples from these sources which include fossil fuel-fired steam generators, home heating stacks, municipal incineraters, Portland cement plants, and coke-oven plants. Only a few collection methods are described here.

The EPA Method-5[13] is designed to determine the particle emissions from stationary sources under isokinetic conditions. The sample is isokinetically withdrawn from the stack by means of a heated probe (minimum temperature of 250°F at the exit end) to prevent condensation of vapors and passed through a heated (minimum temperature of 225°F) filter holder containing a preconditioned glass-fiber filter. The particulate-filtered gases are subsequently passed through four impingers, the first two containing 100 ml water, the third one empty, and the fourth one containing 200 g silica gel. All the impingers are cooled in an ice bath. The exit gases from the last impinger pass through a dry test meter for recording the flow rate. The flow rate is adjusted by the valves attached to the air pump(s).

Doubts have frequently been expressed regarding the collection of volatile organic compounds by the EPA Method-5 sampling procedure described above. The adsorbent sampler method developed by Jones et al.[14] utilizes a Tenax bed to adsorb these volatile organic compounds and reduce the sampling errors. The stack gases, according to this method, are sampled isokinetically by a sampling probe and passed through a heated filter as in EPA Method-5. After leaving the hot filter, the emissions are cooled in a glass coil (120 by 0.8 cm) of the adsorbent sampler and, then, pass through a Pyrex frit and into a cylindrical column of Tenax bed (7 by 3 cm) containing 12 g of the resin. The flow rate through the adsorbent sampler is typically 14 liters/min. The cooling coil and Tenax adsorbent are maintained at a constant temperature by means of a thermostated circulating water bath. The gases leaving the sampler are drawn through an aqueous impinger, a silica gel trap, and a dry gas meter by means of a vacuum pump as in EPA Method-5.

Laboratory validation studies for the recovery of a few POM from spiked samples by the above adsorbent sampler method is presented in Table 4-1.

Table 4-1. RECOVERY OF POM FROM ADSORBENT SAMPLER METHOD[14]

POM	Recovery from test #1	Recovery from test #2	Recovery from test #3
Pyrene	91±3	98±4	104±4
Chrysene	90±5	92±5	106±5
Perylene	91±4	105±5	102±6
Benzo[ghi]perylene	101±10	106±10	103±7
Coronene	80±7	92±8	100±14

Although the absolute collection efficiency is difficult to determine under actual field sampling conditions, a comparison of the total POM loading between adsorbent sampler method and EPA Method-5 was made by Jones et al.[14] Field studies with a 50-hp oil/gas fired boiler and effluents from carbon black manufacturing facilities showed total POM loading of 55.2 and 124 μg/m^3, respectively, with adsorbent sampler and 4.2 and 56.5 μg/m^3 with EPA Method-5. The higher collection efficiency for the adsorbent sampler is apparent from this study. In addition, the adsorbent sampler method reduces the sampling time by as much as 50 percent and demonstrates superiority in its performance for collecting POM from combustion systems burning higher sulfur fuel at a relatively high particle loading.

As a phased approach to environmental sampling, a Source Assessment Sampling System (SASS) has been developed by EPA's Industrial Environmental Research Laboratory.[15] Elements in the SASS train are designed to separate source samples into size fractionated particulate, organic vapors, and inorganic components. The sample from the stack is introduced into this sampling system by a stainless steel inlet nozzle and a heat controlled stainless steel probe at a flow rate of approximately 4 cfm. The sample from the probe enters three cyclones with a backup filter to give four sizing intervals of ~10 μm, 10-3 μm, 3-1 μm, and less than 1 μm. The temperature of the cyclones and filter is maintained at 205°C. After particulate removal, the hot sample gas is cooled to 20°C and passed through the XAD-2 sorbent. The condensate passing through the XAD-2 is collected separately.

To trap the residual volatile inorganic materials, the sample next enters three oxidative impingers cooled in an ice bath. A fourth ice-cooled impinger containing indicating desiccant is used to prevent moisture from entering the pump. A continuous sampling time of approximately five hours is required for collection of acceptable sample size.

It should be mentioned that the SASS train has been used for a limited field validation study. In one study[15] conducted with doped fuel-fired boiler, the recovery of four volatile metals was found to vary between 33% to 75%. Another field evaluation study conducted with the SASS train proved that the particulate concentrations determined by this method compared very well with EPA Method-5 in terms of both precision and accuracy.[16] Although the reproducibility of the SASS train for POM collection was not individually evaluated in this study, organic materials collected by two SASS trains agreed well in quantity and composition.

Recent studies[17] have demonstrated that the stainless steel gas cooler and XAD-2 cartridge of the SASS train may cause sample contamination with the incoming gas stream. Replacing stainless steel with pyrex glass appears to have obviated this problem.

A simple non-isokinetic sampling procedure has been described by Parson and Mitzner[18] in which Pyrex glass tubing containing Tenax resin is directly inserted into the stack. A portable pump is used to draw the sample through the Tenax column at a rate determined by a rotameter. However, the serious drawback with this system is its obvious inability to quantitatively adsorb all the POM at elevated stack temperatures.

A recent study conducted by Adams et al.[19] to characterize the behavior of different resins used as the sorbent trap showed that XAD-2 has a greater volumetric and weight capacity than Tenax GC resins. Therefore, XAD-2 is preferred as a sorbent trap for collection of POM. A modified EPA Method-5 train with a sorbent trap operating for four hours is capable of collecting all materials boiling above 190°C when using XAD-2 and above 240°C when using Tenax GC traps. Both materials efficiently collect POM, but the XAD-2 will have a much greater capacity for the lower boiling compounds. A source sampling train using XAD-2 as the adsorbent has been described earlier.

4.2.3.2 Fugitive emissions from road-way dust--Owing to abrasions of tar and bituminous road surfaces, particles are liberated which contain carcinogenic POM. It is, however, important to distinguish the contribution of vehicular traffic towards the overall POM emissions near a highway. Emission from exhausts, burning or spillage of lubricating oils, and tire wear are factors arising from vehicular traffic which cause POM emission in the air. One way to evaluate the POM emissions arising from abrasion of road materials alone is to make comparative measurements alongside a highway section of tar-asphalt and one of concrete, both subjected to an identical traffic load.[20] The POM emissions due to road abrasion are also dependent on seasonal variation.[20]

For collecting road abrasion samples according to particle size, an Andersen sampler has been used by Ciaccio et al.[21] When properly calibrated, the sampler can be used to collect particles on stages 2 through 7, representing aerodynamic diameters from 0.43 to 7.0 µm which are purported to be deposited in the three compartments of the lung.

The Model 21-000 Andersen sampler, containing stages 0-7 having 1-mil thick polypropylene circle on each stage, is conditioned at a controlled temperature and humidity and the polypropylene circles on each stage are weighed and placed back on the stages. The sampler is assembled and the collection started at an appropriate rate. At the end of the sampling period, the polypropylene circles containing the collected particles are conditioned and analyzed for determining the particle size distribution.

For collecting total particulate matter, a Hi-Vol sampler with the filter holder containing a flash-fired glass-fiber filter has been used.[21] The flow rate during sampling can be adjusted to a suitable value for efficiently collecting the particulate matter.

4.2.4 Ambient Air

The sampling of POM in ambient air is usually performed with the objective of determining the particle size distribution and nature and concentrations of individual components at various points in the environment. The selection of sampling sites play important roles in sample collection. Sampling sites are determined to evaluate the following: (1) characterize the rural or urban background levels, (2) assess the health hazards to people in the vicinity, (3) determine source effects, and (4) establish transport mechanisms. Reference should be made to the available sources[22,23] for detailed treatment of these aspects in the selection of sites.

In addition to the selection of a preferable method and site, certain other factors must be adequately taken into consideration during ambient sampling. The height of the sampler intake above ground level and the local topography and climate each has influence on the data obtained. For example, the influence of summer-like temperature on losses of benzo[a]pyrene from airborne particles has been studied during real high-volume atmospheric samplings.[24] Occurrence of seasonal variation in specific surface areas and densities of suspended particulate matter has been observed.[25] The effects of wind on the collection efficiencies of particulate matter has been demonstrated by Ogden and Wood.[26] Other factors, such as sampling rate and time, are also important in the overall sampling strategy. The total amount of samples to be collected usually depends on the sensitivity of the analytical method intended to be used for the determination of individual components.

The method for collection of POM from ambient air will be divided into two sections depending on the objective of the sampling.

4.2.4.1 Collection of total air particulate matter--Air particulate matter is usually collected on flash-fired glass-fiber filters using a high volume air sampler. The sampler consists of three units: (1) the face plate and gasket, (2) the filter adapter assembly, and (3) the motor unit. The sampler must be capable of passing environmental air through an approximate 400 cm^2 area of a clean 20.3 x 25.4 cm glass fiber filter at a rate of at least 1.13 m^3/min. The glass-fiber filters should have collection efficiencies of at least 99 percent for particles of 0.3 micron or larger in diameter. The motor must be capable of continuous operation for at least 24 hour periods. It is important that the sampler be properly installed in a suitable shelter to protect it from extreme weather conditions and debris. For measuring the air flow rates through the sampling unit, it must be provided with either a calibrated flow meter (rotameter) or a gauge. The details of this method are given by Sholtes et al.[27]

On the average, when a high-volume sampler is located in an urban area, it will collect approximately 250-350 mg of particulate matter while sampling 2000-2400 m^3 of air during a 24 hour period. Of the total amount of the particulate matter, approximately 10 percent, that is, 25 mg, will be the organic fraction.[28] The quantity of organic fraction generally needed for the analysis of POM fraction depends on the sensitivity of the analytical method used. However, to obtain the needed amount of organic fraction it may become necessary to pool together organic fractions of several individual high-volume air samples from a single monitoring site.

POM are present in polluted air sorbed onto airborne particulate matter, usually characterized as primary and secondary. Primary particulate matter is found in sizes between 1 μm and 20 μm.[29] Secondary particulate matter ranges in size from molecular clusters of the order of 5 nm to particles with diameter as large as several micrometers.[29] Very fine particles that cannot be retained on fiberglass filters act like a gas or vapor in that they follow fluid streamlines and are subject to Brownian motion. For collection of this gaseous phase various adsorbents have been used.

In one of the methods,[30] polyurethane foam has been used as adsorbent. An aluminum sampling probe used to collect suspended particulate matter consists of a piece of tubing with a grid for holding a glass-fiber filter and two cylindrical polyurethane plugs (8.5 x 6.0 cm). The plugs located behind the glass-fiber filter prevents loss of volatile fraction of POM. A similar method has been used by Fox and Staley[31] for collecting both particulate and gas phase components from atmospheric samples.

Schuetzle et al.[32] designed an apparatus for simultaneous sampling of gaseous and particulate air pollutants. An adjustable one-stage impactor has been used for sampling aerosols greater than 1-2 μm in diameter and is followed by a glass-fiber filter for collection of particles less than 1-2 μm in diameter. A properly conditioned Chromosorb-102 support is used to trap gaseous air pollutants.

A sampling system using only an adsorbent has also been used.[33] It is a simple system consisting of a vacuum pump, a flow measuring and regulating device, and a sample tube containing the adsorbent. The sample tubes are 110 by 10 mm and contain 4 ml of conditioned Tenax GC held in position by plugs of silanized glass wool.

The usefulness of the adsorbent in collecting POM not otherwise retained on the glass-fiber filter is shown by Krstulovic et al.[30] Table 4-2 shows that the amount of POM collected on the adsorbent depends both on the nature of POM and the location of collected sample.

4.2.4.2 Distribution of particle size--An assessment of the POM content of the polluted atmosphere with respect to size of particles in an aerosol often becomes necessary. Knowledge of the fraction of the air particulate matter which can cause deposition in the various compartments of the respiratory system is of prime importance since some of the POM are proven carcinogens.

Table 4-2. AMOUNTS OF POM COLLECTED ON GLASS-FIBER FILTER AND POLYURETHANE FOAM [30]

Compound	Location A		Location B		Location C	
	Amt. on filter ng/1000 m^3	Amt. on adsorbent ng/1000 m^3	Amt. on filter ng/1000 m^3	Amt. on adsorbent ng/1000 m^3	Amt. on filter ng/1000 m^3	Amt. on adsorbent ng/1000 m^3
Naphthalene	248.0	100.7	31.1	27.9	31.8	49.1
Biphenyl	95.7	8.5	11.3	N.D.[a]	N.D.	N.D.
Phenanthrene	337.7	5.6	46.6	4.9	4.9	6.4
Fluoranthene	1249.8	281.3	N.D.	165.4	159.4	N.D.
Benzo[a]pyrene	29.7	N.D.	N.D.	3.5	4.2	N.D.
1,2,3,4-dibenzanthracene	806.4	3709.2	102.5	N.D.	N.D.	29.7

[a] N.D.: Not detected.

There is considerable disagreement regarding the aerodynamic sizes which can cause deposition of particulate matter in the three compartments of the lung (see Section 6).

Whitby et al.[34] have presented evidence that the mass distribution of atmospheric aerosols is usually bimodal, with one mode occurring below 1.0 μm and the other mode occurring in the 5 to 15 μm range. A dichotomous sampler for particulates has been designed to collect and fractionate samples into two size ranges.[35] Membrane filters in the two air paths collect the respective samples. However, such a sampling technique has rarely been applied to collect POM from air particulate matter.

The experimental determination of particulate distribution is frequently done by five-stage Andersen Hi-Vol cascade impactors.[36] The first four stages of the sampler comprise the fractionating head, while the fifth stage is a backup filter positioned between the fractionating head and a standard Hi-Vol air sampler. When operated at a flow rate of 20 cfm, the sampler fractionates suspended particulate matter into five aerodynamic size ranges according to certain cut-off diameters.

4.3 DESORPTION OF POM FROM COLLECTION MEDIA

A number of methods are available for the desorption of the collected POM from the filter and adsorption traps used for sample collection. Three methods most commonly used are: (1) extraction, (2) thermal desorption, and (3) vacuum sublimation. Each of these methods has been individually discussed.

4.3.1 Extraction

In the extraction method, collected POM are desorbed from the filtering medium by means of a solvent. Depending on the technique used, the extraction method can be subdivided into three distinctly different groups: (1) solvent extraction, (2) mechanical disruption, and (3) HF acid dissolution.

4.3.1.1 Solvent extraction--The principle behind solvent extraction is to dissolve the collected POM from the filtering medium by digesting in a suitable solvent. The usual method involves refluxing of the filter in a Soxhlet extractor for a certain length of time. A wide variety of solvents have been used for this purpose. These include acetone,[44-47] benzene,[6,28,47-49] cyclohexane,[47,49,51-53] methanol,[47,49] tetrahydrofuran,[19,30,47] methylene chloride,[28,47,55,56] chloroform,[57] pentane,[22] carbon disulfide,[49] and a methanol-benzene mixture.[58,59] Potthast and Eigner[60] used propylene carbonate as a solvent for extraction of POM from meat products. They claim that the POM are more soluble in this solvent than any other. The selection of a particular solvent or a mixture of solvents is based on the efficiency of extraction, selectivity in dissolution, time required for extraction, and the reproducibility of extraction. Liberti et al.[47] carried out the extraction process with various solvents, such as cyclohexane, benzene, dichloromethane, tetrahydrofuran, acetone, and methanol. Not only was the extraction yield for PAH maximum with benzene but benzene also showed selectivity towards POM extraction. The extraction time was found to be reasonable and a 10-hour period was found adequate for complete extraction. Experiments carried out with cyclohexane showed a poorer efficiency and a much longer extraction time. Based on these data, these authors recommended benzene as the solvent for POM extraction.

Cautreels and Cauwenberghe[49] also made a comparative study of the various solvents for extraction of organic compounds from an artificial aerosol model which may not be representative for real atmospheric suspended matter. Their study of various solvents, e.g., benzene, carbon disulfide, cyclohexane, hexane, and methanol is presented in Table 4-3.

Table 4-3. COMPARISON OF THE EXTRACTION YIELD OF POM WITH DIFFERENT SOLVENTS[49]

Compound	% extraction yield			
	Benzene	Carbon disulfide	Cyclohexane	Hexane
Phenanthrene	96	98	46	69
Fluoranthene	99	100	52	58
Pyrene	96	97	52	62
Benzo[b]fluorene	101	99	31	28
Benz[a]anthracene	102	106	68	--[a]
Benzo[a]pyrene	75	65	6	--
Dibenz[a,h]anthracene	43	39	--	--
Benzo[ghi]perylene	39	40	--	--

[a] Below the detection limit.

Although the yields of extraction of POM were found to be 10 to 20 percent higher with methanol than with benzene, the latter solvent showed more selectivity towards POM than methanol. The quantitative extraction time was determined to be 8 hours. Both cyclohexane and hexane proved unsuitable for the extraction of POM. These authors[49] also showed that larger errors could occur during the extraction of particulate matter than during the analytical method itself.

It should be mentioned that due to the nature of the artificial sample the extraction yields of PAH as reported by the above authors may have significance only for comparison of various solvents. The absolute extraction yields from natural samples may vary considerably from these results. Various laboratories have reported a much higher cyclohexane extraction efficiency for BaP from glass-fiber filter samples.[50]

4.3.1.2 Mechanical disruption--The widely used Soxhlet extraction is time consuming, decomposes some of the pollutants, and lacks good precision.[61] In order to avoid these errors, a mechanical disruptive technique which makes use of a specially designed blender has been used by Bove and Kukreja.[62] The method has been claimed to be rapid and precise for the extraction of organic contents from glass-fiber filters. A superior procedure has been described by Golden and Sawicki.[52] In this procedure, the sample is suspended in cyclohexane and subjected to ultrasonic waves at room temperature. Silica powder (5 micron size) is added to adsorb polar co-extractives. At the end of sonification, the extract is filtered on a sintered glass filter. The entire method has been evaluated by Sawicki et al.[63]

The recovery of POM by the sonification method was found to be 95 to 98.2 percent. A comparison of the recovery of aromatic compounds between ultrasonic and Soxhlet method showed that the ultrasonic method extracted 49 percent of the total particulate matter collected on glass-fiber filters with a relative standard deviation of ±1.33 percent compared to 30 percent for the Soxhlet extraction with a relative standard deviation of ±26.1 percent of the total extractables. Not only the extraction efficiency but the reproducibility of the ultrasonic method was superior to the Soxhlet method.

Disadvantages with the sonification method lies in the fact that the ultrasonic extraction must be done in a way that avoids an unacceptably high noise level.

4.3.1.3 Hydrofluoric acid dissolution--This technique utilizes the ability of HF acid to chemically dissolve the glass-fiber filters, leaving the organic residue of the filter paper free to extract quickly with an appropriate solvent. For samples collected on more than one 8 by 10 inch filter, the extraction time was about 30 minutes.

Comparison of the HF method with the 6-hour Soxhlet extraction technique is given in Table 4-4.

Table 4-4. COMPARISON OF HF EXTRACTION WITH SOXHLET EXTRACTION[64]

Sample number	weight of extractibles, mg	
	HF Method	Soxhlet Method
1	2.7	3.4
2	2.4	2.0
3	2.6	1.9
4	3.0	3.3
5	2.3	2.6
6	2.3	2.4
7	3.4	2.6
8	2.7	2.2
9	3.5	3.2
10	3.0	3.1
11	2.6	2.1
12	2.4	2.8
13	2.5	2.7
Blank	1.0	1.0

Determination of absolute efficiency of extraction for three POM by the HF method showed high percent recovery values (97.7 to 100 percent). It was also proven that a Lewis acid such as HF did not cause ring and/or side chain rearrangements for alkyl-substituted aromatics.[64] The authors suggested that the new method should prove highly useful for enriching POM content collected on glass-fiber filters. However, this procedure lacks general applicability since some of the collected organics would react with HF and the hazards associated with the handling of highly reactive HF acid are considerable.

4.3.2 Thermal Stripping

The extractive methods discussed above require that the extract be concentrated and usually only a small aliquot of the concentrate be used for analysis, e.g., gas chromatography. This results in overall reduced recovery and sensitivity. Gas-phase extraction, however, is more efficient. According to this method the POM trapped on the collecting media are thermally stripped directly into the separating and detecting device. Since the method involves no evaporative concentration, it eliminates the corresponding loss factor. Another advantage of this method is that the whole sample collected (instead of a fraction of it) can be used for a single analysis, thereby increasing the sensitivity of the method and eliminating the need for collecting large amounts of sample.

Burchfield et al.[65] used this method for the analysis of POM from airborne particulate matter. The Hi-Vol filter was ground to about 20 mesh in a Wiley mill. The samples were then loosely packed into a special stripper tube and the organic components desorbed by a stream of nitrogen at 300°C. The desorbed compounds were trapped in a cold column containing the same packing that would subsequently be used for GLC. Although a minimum of 10 hours was required, stripping was carried out overnight. Due to the long stripping time, variations in gas flow rates between the tubes were unimportant. The compounds trapped in the cold column were finally heated and the compounds were separated on the main column.

A very similar method employed by Bertsch et al.[33] consisted of heat desorption of organic compounds from a porous polymer (Tenax GC) phase instead of a glass-fiber filter-trapped phase. The desorbed compounds were trapped on Emulphor ON-870 cooled at dry ice or liquid nitrogen (N_2) temperature. The advantage of this method was the substantial reduction of stripping time from 10 hours to 15 minutes only.

A slight modification of the above methods which eliminates the cold trap utilizes direct injection of the heat-desorbed components onto a packed column by way of a gas-sampling valve.[18] This method is simpler than the other two stripping methods since it eliminates the special accessory equipment needed for the cold trap.

There are several disadvantages with the thermal stripping methods. Even though the recovery of the adsorbed compounds by thermal stripping methods may be high, data showing this effect are still lacking. This is primarily due to the difficulty in introducing internal standards in the system. That the high temperature used during the thermal desorption process does not cause any rearrangement and/or decomposition remains to be established. Since the injection technique to the final gas chromatographic phase cannot be instantaneous, it is bound to broaden some peaks resulting in resolution problems. Finally, replicate injections of the same sample is not possible with this technique.

4.3.3 Vacuum Sublimation

This technique for the extraction of atmospheric pollutants has been developed by Japanese researchers. A more recent apparatus for extracting seven particulate samples by one operation is given by Matsushita et al.[66]

The extraction apparatus used is constructed with a rotary pump, a MacLeod manometer, a connector, vacuum sublimation tubes, sublimation flasks and an electric furnace. Seven particulate samples are put in seven sublimation flasks connected with their respective sublimation tubes. These tubes are attached via a common connector to a manometer and a rotary pump. After cooling the sublimation tubes with ice water, these are evacuated to 0.005-0.001 mm Hg. The sublimation flasks are next heated to 310-315°C. POM sublimed from the particulates are deposited on the cold part of the sublimation tube. At the end of 40 minutes of sublimation, the deposited sublimates from the individual tubes are dissolved in a small amount of benzene.

The recovery and reproducibility of the above method was evaluated[66] with spiked samples and for three POM tested the recovery varied between 97.4 and 98.1 percent and the maximum coefficient of variation was 2.13 percent (relative standard deviation). A comparative study between this method and ultrasonic extraction method proved that they both have almost identical recoveries for BaP from airborne particulate samples.[66] However, studies conducted by Monkman et al.[69] contradicted these findings.

The vacuum sublimation technique is faster than Soxhlet extraction and is routinely used by Japanese researchers[41,67] for extraction of POM from Hi-Vol glass-fiber filters. It has also been recommended by Schultz et al.[68] as a routine method for a limited POM analysis.

4.4 CLEAN-UP AND SEPARATION

The next step following the desorption of POM from the collection media consists of clean-up and separation of these compounds from undesirable impurities and from one another. Of course, the extent of clean-up and separation will depend on the nature of the collected sample. This section deals with the various methods used for this purpose. Depending on the method employed, the clean-up and separation process can be divided into: (1) Solvent Partitioning, (2) Column Chromatography, (3) Paper Chromatography, (4) Thin Layer Chromatography, (5) Gas Chromatography, and (6) High Pressure Liquid Chromatography. The individual processes are dealt with separately.

4.4.1 Solvent Partitioning

This is one of the oldest methods used for the isolation of POM as a group from the bulk of impurities. According to this method, the POM in a certain solvent are allowed to distribute in another immiscible solvent which has either higher or lower distribution coefficient for the POM than the original solvent. In the former instance, the POM are extracted in the second solvent, and in the latter instance, the impurities accumulate in the second solvent leaving the first solvent relatively free of impurities. A variety of solvents which have been used for solvent partitioning are: (1) methanol-water, (2) tetramethyl uric acid in methanol, (3) acetonitrile, (4) dimethyl formamide, (5) nitromethane, and (6) dimethyl sulfoxide.

4.4.1.1 Methanol-water--In this solvent partitioning step originally proposed by Hoffmann and Wynder,[70] the POM in cyclohexane solution are allowed to partition in methanol-water solution. The polar impurities in cyclohexane solution preferentially distribute themselves in the more polar methanol-water phase, leaving the cyclohexane phase containing the POM relatively free of impurities. It should be mentioned that a single partitioning step like this is only a part of several partitioning or other separatory steps in the overall clean-up scheme. However, methanol-water phase has been used by a number of other authors[71,73] as a preliminary step for getting rid of the polar components from the POM fraction.

4.4.1.2 Tetramethyl uric acid in methanol--A solvent system incorporating tetramethyl uric acid in methanol as a selective complexing agent has been used to separate several POM. Distribution coefficients for a number of POM in this solvent system have been given by Mold et al.[74] However, this solvent system lacks wide applicability.

4.4.1.3 Acetonitrile--Haenni et al.[75] determined the distribution of POM between n-heptane and acetonitrile and found low distribution ratios. These authors concluded that this solvent is not very effective in concentrating POM compounds.

4.4.1.4 Dimethyl formamide--This solvent was found to be much more efficient for the removal of POM from heptane phase.[75] Consequently, this solvent has been used by other authors[9,73] for extracting POM from cyclohexane phase as well.

4.4.1.5 Nitromethane--The partition coefficients of POM between nitromethane and cyclohexane was determined by Hoffmann and Wynder[12] and varied between 4.4 and 1.65 for a number of POM tested. The same partition coefficients determined between nitromethane and other aliphatic solvents[75,76] showed values quite similar to that determined by the above authors. This solvent has been used by a number of authors, particularly for the clean-up of automobile exhaust samples.[53,55,71,77]

4.4.1.6 Dimethyl sulfoxide--The first evaluation of this solvent as a partitioning medium for POM was made by Haenni et al.[75] A comparison of four solvents, e.g., acetonitrile, nitromethane, dimethyl formamide, and dimethyl sulfoxide, showed that the last solvent was much more suitable on the basis of distribution coefficients and selectivity for POM compounds.[75] Comparative study of the percent recovery between two widely used solvents made by Acheson et al.[78] is given in Table 4-5.

Table 4-5. EFFICIENCIES OF SOLVENT-PARTITION PURIFICATION PROCEDURE[78]

Compound	% Recovery		Compound	% Recovery	
	Nitromethane	DMSO		Nitromethane	DMSO
Fluoranthene	48-83	92-96	Benzo[a]pyrene & Benzo[e]pyrene	33-36	95-99
Pyrene	38-42	94-96	Perylene	< 10-24	84-90
Benz[a]anthracene & chrysene	50-64	90-92	Indeno[1,2,3-cd]-pyrene	41-49	97-100
Benzo[k]fluoranthene	54-59	91-94	Coronene	10.0-44	82-93
Benzo[ghi]perylene	15.8-45	90-91			

From their investigations Acheson et al.[78] conclude that DMSO is the best extractive medium for POM compounds. Other investigators[79,80] have effectively used this solvent for isolating POM compounds from impurities.

It should be mentioned that in a complex mixture it often becomes necessary to first fractionate the POM extract into acid, basic, and neutral fractions prior to further clean-up procedures.[55,77] In this method, the POM mixture in a suitable solvent is extracted with an aqueous alkaline solution to separate the acidic components. Subsequent extraction with an aqueous acid solution separate the basic components from the neutral fraction. By carefully controlling the acidic and basic strength of the aqueous solution, the individual fractions can be further subdivided into groups of compounds with varying acidic and basic strength. By carrying out the extraction with

1N sodium hydroxide or HCl, Cautreels and Cauwenberghe[49] found that on the average 99 percent of n-paraffins stayed in the neutral fraction. About 90 percent of neutral polyaromatics was found in the neutral fraction and 10 percent in the acidic fraction. The yield of other neutrals, e.g., phthalate ester, was about 96 percent in the neutral fraction. The basic fraction gave an average of 75 percent yield for the acidic compounds. The results obtained from the isolation of some selected basic compounds, however, showed very poor recovery.

4.4.2 Column Chromatography

This is the most widely used separation method for the POM. A large variety of adsorbents, such as (1) alumina, (2) silica gel, (3) Florisil, (4) cellulose acetate, and (5) gel materials, have been used for separation. Adsorbents with uniform particle size, 60-80, 80-100, and 100-120 mesh and column diameter:length ratios of at least 1.25 are suggested. The laboratory temperature should be kept reasonably constant and the solvent used for elution should be of highest purity. Maximal separation can be achieved by slowly and evenly increasing the polarity of the eluting solvents. The hydrocarbons are eluted from the column in the following order: aliphatics, olefins, benzene derivatives, naphthalene derivatives, dibenzofuran fraction, anthracene fraction, chrysene fraction, benzpyrene fraction, and coronene fraction.[28] The factors that affect the chromatography of the organic fractions are: (1) the retardation volume, i.e., the volume of eluent passing through the column per gram of adsorbent before the substance in question leaves the column; (2) the volume spread of the eluted substances, i.e., the volume of eluent in which the substance is found; and (3) the volume separation, i.e., the volume of eluent separating one eluent substance from another.[28] These factors can be affected by three variables: (1) percentage of water in the sorbent, (2) percentage of polar solvent in the solvent mixture, and (3) possible miscellaneous factors, e.g., the composition and weight of the organic fraction.

4.4.2.1 Alumina--Alumina is available in various grades, sizes, pH and activity. For the separation of neutral POM fractions, Woelm Neutral Alumina is generally used. The sample:adsorbent ratio varies between 1:100 and 1:1000. The alumina is deactivated with 13.7 percent water prior to use.[84] Elution of the adsorbed compounds is done by gradually increasing the polarity of the solvent system. Ether-pentane,[85] cyclohexane-ether,[46] cyclohexane-benzene,[86] and benzene-methanol[87] are some of the solvent systems used by various researchers. Some investigators[46] have successfully separated PAH on very long alumina column. However, such separation procedures need automatic fraction collectors to collect a number of eluting fractions.

The main disadvantage of alumina column separation is that it is very time consuming, sometimes causes decomposition of compounds on the column (particularly polynuclear aromatic amines), and good reproducibility is often difficult to achieve.

4.4.2.2 Silica gel--It is difficult to get silica gel with a standardized preparation. Material of 100-200 mesh or Davison grade 12 or equivalent is found suitable for chromatographic purposes.[28,71] The sample:adsorbent ratio varies between 1:50 and 1:500. Some investigators used silica gel with 3 to 5 percent moisture content,[47,57] while others used the dried silica gel[51,71] without deactivation with water. The elution of adsorbed compounds is usually done by benzene[51,57,71] or hexane-benzene.[47]

It should be recognized that silica gel is normally used in combination with other adsorbents or other separation methods and is rarely used as the sole separation process. A separation method using both alumina and silica gel chromatography was used by Liberti et al.[47] The average percent recovery for 9 PAH compounds was found to be 77.2 percent. The individual recoveries as reported by these authors are listed in Table 4-6.

Table 4-6. RECOVERY OF PAH BY LINEAR ELUTION ADSORPTION CHROMATOGRAPHY[47]

PAH	% Recovery from alumina & silica gel chromatography
Fluoranthene	68.8
Pyrene	82.0
1-2 Benzanthracene	77.6
Chrysene	82.1
3-4 Benzofluoranthene	82.4
Benzo[a]pyrene	80.5
Benzo[e]pyrene	80.5
Indeno[1,2,3-cd]pyrene	90.6
Benzo[g,h,i]perylene	90.3

Column chromatography with silica gel suffers from all the disadvantages normally encountered with alumina. The relatively slow flow rates of solvents through silica gel causes additional problems.

4.4.2.3 Florisil--Column chromatography using Florisil is not as widespread for the separation of POM as the other two methods already described. Activated Florisil, 60-100 mesh, have been used for fractionation of PAH compounds[77,87] and also for isolation of PAH compounds as a group from other impurities.[79,80] The eluting solvent for this sorbent is usually hexane-benzene[87] or benzene alone.[80] The advantage with Florisil column is that PAH separate rather well, despite a short elution time. To prevent photo-oxidation during chromatography, the Florisil column should be protected from light.

4.4.2.4 Cellulose acetate--Although cellulose acetate is a promising sorbent for separation of POM, its use as a column chromatographic material is very limited. Griest et al.[79] have reported using this technique for the purification of PAH.

4.4.2.5 Gel filtration--Wilk et al.[88] were the first to use this technique for the separation of POM. Others have since found the system to be valuable for separation of PAH from automobile exhausts,[14,89] air particulate matter,[55,90] cigarette smoke,[73] and biological samples.[90,91] Separation of neutral aza-arenes has also been achieved by this technique.[92] The separation is best achieved isothermally on Sephadex LH-20 (ratio, about 1:1000) columns with 2-propanol as solvent at a flow rate of 6-7 ml/min. Gladen[89] determined the recovery efficiencies of 8 PAH by this technique which is shown in Table 4-7.

Table 4-7. RECOVERY OF PAH BY SEPHADEX LH-20 CHROMATOGRAPHY[89]

Compound	% Recovery
Anthracene	101
Phenanthrene	98
Pyrene	94
Fluoranthene	102
Benz[a]anthracene	100
Benzo[a]pyrene	97
Perylene	98
Benzo[g,h,i]perylene	98

Although gel filtration is free from most of the drawbacks of other column chromatographic methods, its main disadvantage is that it takes a long time (1 to 3 days) for separation.

4.4.3 Paper Chromatography

Paper chromatographic methods are used for the separation of individual fractions and are usually preceeded by a preliminary separation by column chromatography. For optimum separation, the commonly used paper is acetylated cellulose[70,81] and the chromatography is carried out by ascending technique. A slight modification of the paper by impregnating with 10 percent liquid paraffin has also been used for chromatographic separation.[82] Developing is done with a variety of solvent mixtures. These include toluene-methanol-water (1:10:1), methanol-ether-water (4:4:1), methanol-chloroform (3:1), ethanol-toluene-water (17:4:1), and ethanol-benzene-water (12:6:1).

In modern separation techniques paper chromatography is rarely used. There are several reasons for this. The time required for separation, difficulty in getting reproducible papers, inadequate resolution of compounds, and the non-quantitative nature of analysis are disadvantages for this technique.

4.4.4 Paper and Thin-layer Electrophoresis

Separation of basic aza-arene by this technique was accomplished by Sawicki.[83] However, this method has restrictive use for reasons similar to the paper chromatographic technique.

4.4.5 Thin-Layer Chromatography

This technique is quick, inexpensive, reasonably reproducible, and one of the better methods for the separation of isomeric compounds. With the availability of in situ scanning technique (thin-layer scanner), this method has been used for quantification of POM with reasonably good accuracy. A number of materials have been used as sorbent materials for the plates. These are: (1) silica,[47,76,96,97] (2) alumina,[95,99,100] (3) cellulose,[83] (4) cellulose acetate,[68,90,93-95] and (5) polyamide.[101] A number of modifications including silinazation of silica gel[102] and channel thin-layer chromatography[103] have been proposed. In the latter procedure, the components which need to be fractionated on a thin-layer plate are made to flow into narrow development channels in order to prevent the spreading of the chromatographic spots.

Both PAH and polynuclear aza-heterocyclic compounds have been separated by TLC method. A large number of variations of TLC procedure, such as variation of developing solvents, use of a mixture of sorbents, developing in one or two dimensions, have been used to affect better resolution of the components. The effect of solvent variations on cellulose acetate TLC has been discussed in detail by Woidich et al.[95] Although alumina-cellulose (2:1) has been used as a composite adsorbent,[98] the best resolution is obtained when two-dimensional TLC on a composite plate of aluminum oxide-40 percent acetylated cellulose (2:1) is used.[104,105] The percent recovery of a few isomeric arenes separated by this method[105] is given in Table 4-8.

Table 4-8. RECOVERY OF VARIOUS PAH DURING THIN-LAYER CHROMATOGRAPHY[105]

Compound	% Recovery	Compound	% Recovery
BeP	86.0±5.3	Benzo[ghi]perylene	88.7±4.0
BaP	85.2±5.1	Naphtha[1,2,3,4-def] Chrysene	87.9±5.5
BbF	92.1±3.5		
BkF	91.2±3.7	Benzo[rst]pentaphene	91.8±4.2
Perylene	90.4±4.6	Dibenzo[b,def]chrysene	89.8±3.9
Dibenzo[def, mno] chrysene	90.9±3.9	Naptho[2,18-gra]naphthacene	90.7±5.1
		Dibenzo[def,b]chrysene	84.1±6.2

A large number of aza-heterocyclic compounds found in urban airborne particulates and in air pollution source effluents have been separated by TLC procedure. The basic fraction from a coal-tar-pitch sample when subjected to a two-dimensional TLC separation on silica gel-cellulose (2:1) with pentane-ether (9:1, v/v) and dimethyl formamide-water (35:15, v/v) solvent systems yielded the results shown in Table 4-9.

Two-dimensional TLC separation has also been successfully used[98] during the quantification of acridine, benz[c]acridine, 7H-benz[de]anthracen-7-one and phenalen-1-one in airborne particulate samples.

Table 4-9. AMOUNTS OF AZA-HETEROCYCLIC COMPOUNDS IN A COAL-TAR-PITCH POLLUTED AIR SAMPLE SEPARATED BY TLC[85]

Compound	Concentration in mg/ 1000 m^3 air	Compound	Concentration in mg/ 1000 m^3 air
Acridine	0.870	Indeno[1,2,3-ij]-isoquinoline	0.030
Benzo[f]quinoline	0.420		
Benzo[h]quinoline	0.260	11 H-Indeno[1,2-b]-quinoline	0.190
Phenanthridine	0.020		
Benz[a]acridine	0.200	Dibenz[a,h]acridine	0.010
Benz[c]acridine	0.120	Dibenz[a,j]acridine	0.001

There are several disadvantages with the TLC procedure. Mass spectrometry and liquid chromatography, when applied as additional analytical tools for identification of POM, have demonstrated that the individual spots or bands are sometimes, in fact, mixtures of two or more compounds. Also, the TLC procedure cannot be used when separation of a large number of compounds is required since TLC resolution is limited. Furthermore, during TLC procedure trace amounts of compounds may photodecompose requiring addition of an internal standard to correct for the losses.

4.4.6 Gas Chromatography

The closeness of the chemical structure among some of the POM and their relatively high boiling points demand the stringent requirements of high resolution and thermal stability at high temperature for the chromatographic column packings. Unfortunately, until recently the available liquid phases that were thermally the most stable were barely adequate for the temperature required to separate some of the high boiling POM. However, a large variety of column packing materials have since been used for POM separation. Depending on their mode of operation, gas chromatographic methods used so far can be divided into: (1) packed column; and (2) capillary columns, e.g., surface-coated open tubular column and wall-coated open tubular column.

4.4.6.1 Packed column--A number of packing materials have been used in packed column chromatography. A gas-solid chromatographic phase using Chromosorb 101[115] and GLC (gas liquid) phases using OV-1,[65,106] OV-7,[98] OV-25,[79] polymetaphenoxylene,[93] apiezon L[98,108] apiezon W,[79] SE-30,[86,108,109] SE-52,[57,110] Dexsil-300 GC,[49,65,106,111,113,114] Dexsil-400 GC,[106] Dexsil-410 GC,[106] and nematic liquid crystals[114,116,117] have found their applications as the packing materials.

One of the superior column packing materials available for POM separation is Dexsil-300 or Dexsil-400. The column bleeding characteristic and resolution capability for these packing materials make them highly suitable for POM analysis. The resolution of Dexsil-400 is even better than Dexsil-300.[106] The separation of both PAH and aza-arenes in samples containing coal tar has been accomplished with these packing materials.[106] Dong et al.[118] have used Dexsil-300 column for the separation and quantification of a number of aza-arenes in the basic fraction of airborne particulate matter.

Nematic liquid crystals with the general formula N,N'-bis(p-substituted benzylidene)-a,a'-bi-p-toluidine have been shown to resolve hard-to-separate PAH isomers. Three p-substituted compounds, methoxy (BMBT), hexyloxy (BHxBT), and phenyl (BPhBT) have been investigated.[114,116,117] However, column bleeding at higher temperatures remains a problem with this packing material. For routine separation of benzpyrene isomers, a BMBT packed column operated isothermally at 130°C has been found useful.[114]

4.4.6.2 *Capillary column*--These columns are more advantageous than packed columns due to their ability to affect higher resolution. The number of theoretical plates obtained from such columns may vary between 20,000-70,000.[119] Versamid-900,[102] SE-30,[119] XE-60,[119] SE-52,[11,53,58,72,119,120,123] SE-54,[125] *m*-bis-*m*-(phenoxylphenoxy)-benzene,[121] Dexsil-300,[14] Emulphor ON 870,[33] carbowax-20 polymer,[122] OV-1,[124] OV-17,[9] OV-101,[9,73,91] apiezon L,[47,76] and SP-2100[126] all have been used as packing materials for capillary columns. The most widely used column for separation of POM is SE-52. Cantuti et al.[119] compared several liquid phases and found that a SE-52 column of 50 m length was most effective for POM separation and had a theoretical plate of 40,000. Recent investigations by Bjørseth[125] have demonstrated that capillary columns using SE-54 as the stationary phase have excellent separation efficiency, low column bleed, and long-term stability. Both PAH and aza-arenes have been separated with this packing material.[125] A 40-m capillary column with Versamid-900 as the stationary phase is very effective for the separation of aza-arenes (theoretical plate of 60,000).[102]

Investigations by Lao et al.[127] with Dexsil-300 coating liquid has shown that the separation of POM on surface-coated open tubular column is better than packed columns for the range from fluoranthene to methylchrysene. However, the resolution of the later peaks with longer retention times remains incomplete.

Analysis of POM carried out by wall-coated capillary columns, coated with SE-52 of liquid phase thickness of 0.27 μm showed excellent reproducibility, high sensitivity, and high resolution between individual isomers in comparison to regular packed columns.[128] WCOT columns have been claimed to offer numerous advantages over packed or porous-layer open tubular columns.[128] Some of these are superior resolution, minimum adsorption effect, and high stability.

4.4.7. High Pressure Liquid Chromatography

The ability of HPLC to separate POM compounds not normally accomplished by GLC indicates the great potential of this method for POM analysis. In addition, HPLC offers many advantages over GLC: it operates at much lower temperatures, fraction collection is simple and convenient, the capacity to accept sample is high, and fluorescence spectroscopic detection demonstrate high sensitivity and selectivity for POM compounds.

In HPLC, the mobile phase modifier is the most powerful variable for changing retention times. The choice of mobile phase is dictated by the mode of separation accomplished by the sorbent. In the normal mode, generally hydrocarbon solvents are used as the mobile phase. Any solvent that dissolves POM in fair amounts, is miscible with water, and is transparent at U.V. absorption wavelength (for U.V. or fluorescence detector) can be used as a modifier in the reverse phase-L.C.

It should be mentioned that solvent programming has proven to be a powerful tool in separating the compounds from each other. Several researchers have taken advantage of increased temperature (70 to 80°C) of the column to obtain a similar objective. At higher temperature reduced viscosity of the mobile phase increases column efficiency and decreases relative retention times. The sample capacity is also increased at higher temperatures allowing larger amounts of sample injection. Table 4-10 summarizes the different columns and mobile phases used by various authors for the analysis of POM by HPLC. The most versatile columns for POM analysis operate on the principle of reversed-phase mechanism. One such column containing μ-Bondapak C_{18} has been applied for the separation of both PAH[97] and aza-arenes.[144]

4.5 DETECTION

The different methods of detection with their relative advantages and disadvantages have been individually discussed in the following sections.

4.5.1 Flame Ionization and Electron Capture

When gas chromatography is used for separation and analysis of POM the usual method of detection is either flame ionization detection (FID)[11,53,77,93,108] or electron capture detection (ECD).[77,93,108] One advantage with the EC detector is its greater selectivity towards BaP compared with BeP.[148] However, the high temperature used in the column oven for the separation of higher boiling compounds requires even higher temperature capability for the detector. Most EC detectors do not fulfill this requirement. As a result, most of the recent GC analysis have been performed with FI detectors. The advantage with the flame ionization detector is its wide dynamic range of linear response for most POM.

Table 4-10. HPLC CONDITIONS AND SEPARATION MECHANISMS USED FOR PNA ANALYSIS

Nature of Sample	Stationary Phase	Mobile Phase Mode of Elution	Separation Mechanism	Detection Mode	Reference
Standard PAH solution	120/170 mesh Alumina	n-pentane isocratic at 211¼C	Adsorption	Disc conveyor FID	129
Carbon black	ODS-Sil-X-I	30% H_2O:70% MeOH isocratic at 50¼C	Reversed phase	U.V.	130
Sediment & sea water	Coupled Bondapak C_{18}-μ Bondapak C_{18}	Gradient elution 30% MeOH:70% H_2O to 100% MeOH	Reversed phase	U.V., Fluor.	131
Automobile exhaust	Porasil T	n-heptane isocratic at 51.5¼C	Adsorption	U.V.	132
Standard PAH solution	ODS-Permaphase	65% MeOH:35% H_2O isocratic at room temp.	Reversed phase	U.V.	133
Standard PAH metabolite & solution	ODS-Permaphase	30% MeOH:70% H_2O to 70% MeOH:30% H_2O gradient elution	Reversed phase	U.V.	134
Standard PAH solution	2,4,7-trinitrofluorenone impregnated Corasil I	n-heptane isocratic at 25¼C	Charge-transfer	U.V.	135
Standard PAH solution	3(2,4,5,7)tetranitrofluore-iminopropyldiethyl siloxane	Acetonitrile-Isooctane isocratic	Charge-transfer	U.V.	136
Automobile exhaust condensates	ODS-Permaphase	Linear gradient 20% MeOH:80% H_2O to 100% MeOH	Reversed phase	U.V.	137
Standard PAH solution	Spherosil XOA-400	Isooctane isocratic	Adsorption	Fluor.	138
Standard PAH solution	Corasil-C_{18}	80% MeOH:20% H_2O isocratic	Reversed phase	Fluor.	139

Table 4-10. HPLC CONDITIONS AND SEPARATION MECHANISMS USED FOR PNA ANALYSIS (Cont'd)

Nature of Sample	Stationary Phase	Mobile Phase Mode of Elution	Separation Mechanism	Detection Mode	Reference
Standard PAH solution	Octadecyl trichlorosilanized silica gel	90% MeOH:10% H_2O isocratic	Reversed phase	Fluor.	140
Atmospheric particulate matter	Zorbax-ODS	70% MeOH:30% H_2O isocratic	Reversed phase	Fluor.	31
Atmospheric particulate matter	Zorbax-ODS	65% MeOH:35% H_2O to 1% MeOH/min. gradient	Reversed phase	Fluor.	97
Standard PAH solution	Equal portions of 8.1% dimethylformamide and carbowax-350 on gas chromatograph	Isooctane isocratic	partition	U.V.	141
Standard PAH solution	30% Cellulose Monacetate	Ethanol/$MeCl_2$ (2:1) isocratic	Reversed phase	U.V.	142
Standard PAH solution	100/115 mesh Woelm Basic Alumina	Pressure programmed 95% n-pentane:5% MeOH	Adsorption	U.V.	143
Atmospheric particulate matter	μ-Bondapak/C_{18} & μ-porasil	20-80% MeCN in water at 2%/min. and 1% propanol-2 in hexane	Reversed adsorption	Fluor.	144
Marine sediment & oil samples	μ-Bondapak/C_{18} & μ-Bondapak NH_2	50-70% Acetonitrile in water and hexane	Reversed phase	Fluor.	145
Coal liquefaction products	Cross-linked polyvinylpyrrolidone	Propanol-2	Adsorption	Fluor.	146

4.5.2 U.V. Absorption

This technique is the most widely used method for POM detection.[9,12,28,44,46,84-86,91] The advantages of this technique include the commercial availability of high quality spectrophotometers, the relative insensitivity of the degree of absorption to trace impurities, and the fact that the absorption spectrum of a mixture is usually the sum of the spectra of the components. The latter characteristic offers a crosscheck of the total concentration in a complex mixture determined by other methods. The disadvantages include the requirement of two or more separation steps for reliable identification and the relatively lower detection sensitivity compared to other detection devices.

Although the total spectrum for each POM compound is unique, the individual features are not. Therefore, identification and quantification of a POM compound on the basis of only one peak is unreliable. A computer program utilizing the U.V. spectra has been developed for rapid screening and simultaneous identification of a large number of peaks.[147]

Incomplete separation of individual compounds is a major problem in interpreting U.V. spectra and since the compounds rarely completely separate, questionable results are obtained with U.V. detection of complex mixtures even with a programmed spectral analysis.

4.5.3 Luminescence Analysis

4.5.3.1 U.V. excited luminescence emission--This technique has become well-established as a sensitive and selective technique for POM detection.[51,65-68,71,72,84,91,99] Several groups have found it to be at least an order of magnitude more sensitive than absorption spectroscopy.[138,149] It is also more sensitive (lower detection limit) and less expensive than mass spectral detectors. Mass spectrometers normally have a nanogram detection limit,[150] although integrated ion-current techniques reduce this limit to the subpicogram range.[151]

Since luminescence spectroscopy is nondestructive, individual fractions can easily be collected and subjected to further analysis. Furthermore, this method provides additional selectivity. While many interferring compounds may absorb light, only a few emit in the region of particular POM compounds.

Various modifications of this method have been used to increase the sensitivity and selectivity of the method. Application of low temperature fluorescence and phosphorescence for the characterization of aromatic compounds with enhanced sensitivity has been utilized by Sawicki and Johnson.[152] However, this technique is rarely used at the present time. Room temperature phosphorescence measurements have been made without the problems of typical low temperature sample matrix preparation, yet maintain good sensitivity and selectivity. Phosphorescence is increased by adsorption of the sample on to a rigid matrix-like backing such as filter paper, thereby quenching non-radiative vibrational de-excitation,[153] and by the addition of a heavy atom perturber which increases the spin-orbit coupling between the singlet and the triplet excited states.[154]

The selectivity of fluorescence detection for POM compounds has been increased by characterization at two wavelength combinations, e.g., at 313/360 and 365/445 nm as a double fingerprint.[155] By means of a selective excitation and/or oxygen-quenching method, POM compounds have been quantitatively analyzed without chromatographic separation.[156] Identification of compounds showing minor features in zeroth derivative fluorescence spectra has been accomplished by first and/or second derivative fluorescence and fluorescence modulation techniques.[31] Since derivative spectroscopy deals with the shape characteristics of the spectral absorption rather than the intensity changes, increased sensitivity and selectivity are obtained over conventional absorption spectrometers. The curvature characteristics are often quite large and specific to individual compounds, thus allowing selective analysis of a component in a rather complex mixture. In addition to the scanning mode of operation where a complete spectrum is obtained, the second derivative spectrometer can be operated in a "dwell" mode at a specific wavelength allowing for real-time monitoring of a selected component.

In situ fluorescence detection on a thin-layer plate with a scanner has also been used to increase the detection limit of POM compounds.[80,90,94,95] Fluorescence spectroscopy using variable wavelength detectors has been used to increase the selectivity of detection in the HPLC method.[30,54] Finally, multicomponent analysis by the use of rapid scanning fluorescence spectroscopy interfaced with a computer for data reduction by algorithm programming has been studied.[157]

However, there are quite a few complications with this detection technique. All emission techniques are influenced by repeatability of wavelength settings, scatter of light in monochromators, and random fluctuations in source intensity. Quenching by oxygen is a serious problem and may be very selective. Impurities which introduce intersystem crossing will enhance phosphorescence at the expense of fluorescence intensity.

4.5.3.2 Shpol'skii effect--Detection of PAH compounds by this technique was first demonstrated by Shpol'skii[158] in 1952. The characteristic sharp-line (quasi-linear) luminescence emission spectra obtained by U.V. irradiation of the crystalline matrix of PAH in n-paraffin solvents at 77°K or below have been used for the identification of these compounds. The original n-paraffin solvents used for this study were n-hexane and n-heptane. However, most PAH compounds may not be soluble in n-paraffins. Therefore, Kirkbright and DeLima[159] used 10% (v/v) cyclohexane in n-paraffin as the solvent mixture which was demonstrated not to disturb appreciably the spectrum obtained. From the study of Shpol'skii effect with 23 PAH compounds in several n-paraffin solvents, such as n-hexane, n-octane, and n-decane, these authors also concluded that some PAH produced more well-defined spectra in a particular n-paraffin solvent compared to another.

Although it appears that direct measurement of the intensity of quasi-linear luminescence emission is capable of permitting the quantitative determination of PAH compounds directly, real samples pose several problems. The effects of energy transfer, the inner-filter effect, and experimental variables all cause error in the analysis. The use of combined internal standard - standard additions technique has been demonstrated to yield acceptable results in such samples.[159] This luminescence emission method is also suitable as a "fingerprinting" technique for qualitatively identifying PAH in a mixture.

4.5.3.3 X-ray excited optical luminescence (XEOL)--This method is a slight variation of Shpol'skii effect in that it uses X-ray (1 to 10Å) to obtain quasi-linear fluorescence and phosphorescence-emission from samples frozen in n-heptane matrix at 90°K. D'Silva et al.[160] reported this technique as a method for the detection of nanogram levels of PAH. The suitability of this method in the analysis of real samples remains to be established. However, some of the potential advantages for X-ray over U.V. excitation are: (i) freedom from optical cross-talk between the exciting and luminescence radiation; (ii) larger population of higher energy electron levels, thereby revealing additional lines; and (iii) substantial phosphorescence emission, aiding further characterization from these lines.

4.5.3.4 Sensitized fluorescence--The inherent fluorescence of PAH compounds is greatly enhanced in the presence of certain sensitizers, thereby decreasing the fluorescence detection limit of the former compounds by several orders of magnitude. This technique has been employed as a spot test method for screening environmental samples for the presence of PAH.[161] According to this method, naphthalene as sensitizer is co-spotted with the sample spot on a Whatman #42 filter paper. The dried spot is exposed to light of appropriate wavelength and intensity and any sensitized fluorescence emission is recorded. On filter paper, 10 pg of PAH in a spot can generally be visualized when treated with naphthalene. Experiments with a few real-life samples have shown that the method is specific for PAH with minimum interference from other compounds. However, large concentrations of nitroaromatic compounds have been shown to act as fluorescence quencher in this method.

4.5.3.5 Synchronous luminescence spectroscopy--A recently developed method termed synchronous luminescence spectroscopy, where the excitation and emission wavelengths are simultaneously varied with a fixed wavelength difference of 3 nm between the two, has been proposed[162] for the routine monitoring of PAH in complex samples. The spectra obtained from this technique is simplified with improved resolution between component peaks.

4.5.4 Mass Spectrometry

Mass spectrometry affords a sensitive means of determining the probable identity and relative purity of POM chromatographic fractions. This method has gained wide acceptance for analysis of POM compounds.[32,33,59,73,112-115,120,121] Generally, this has been accomplished by low voltage ionization methods which produce spectra containing mainly singly-charged, intact molecular ions.

In POM analysis, the most widely used methods for sample introduction to the mass spectrometer are direct insertion probe[32,112,151,163] and direct interfacing GC-MS method.[106,115,121,127] The sample ionization is commonly done by electron impaction and the separation of ion beams is accomplished by either low resolution quadrupole instrument or high resolution magnetic scanning equipment. The claimed[113] advantage of quadrupole instrument is: (i) its initial relatively low cost; (ii) ability for straightforward maintenance and repair; and (iii) extremely high-speed linear mass scan, simplifying system control, data logging and spectra interpretation. The disadvantage of the quadrupole method is its low mass resolution and low mass sensitivity at higher masses. Both high resolution[32,151,163,164] and low resolution[106,113,114] mass spectrometers have been used for PAH and aza-arene analysis.

The analysis of complex samples usually produces numerous spectra and the spectrometer generates an enormous amount of data from a single chromatogram. Therefore, the data reduction and quantification requires a computerized data processor with an appropriate programming system. At the end of the GC run, the computer is used to plot a reconstructed gas chromatogram of total ion amptitude versus the spectrum number (ion abundance chromatogram).

Identification of these chromatographic peaks is accomplished by plotting the mass spectrum of a specified peak or by specific ion monitoring (mass fragmentography).

One problem with electron impact ionization is that isomeric POM may give identical mass spectra making unambiguous identification sometimes impossible. Hites and Dubay[165] found that charge-exchange-chemical ionization mass spectrometry can be used to distinguish many isomeric POM. They have established that spectra of POM have characteristic ratios of the protonated molecular ion to the molecular ion when 5 to 10 percent methane in argon is employed as the reagent gas. This technique is not expected to replace electron impact GC-MS, but it should be a useful supplemental tool for differentiating both isomeric PAH and aza-arene.

4.5.5 Other Techniques

Fourier-transform NMR spectroscopy[56,72] has been used for the elucidation of the position of substitution in the benzene ring positions of the POM. Other techniques of detection utilizing matrix isolation Fourier-transform IR[166] and Piezoelectric crystal[167] need considerable improvement to become viable methods for POM analysis.

4.6 ANALYTICAL METHOD USED BY EPA FOR NASN MONITORED SAMPLES

The following method[173] was used for the quantification of BaP from NASN monitored samples collected during the period 1966-1972.

The particulate sample collected on glass-fiber filter was extracted with benzene in a Soxhlet extractor for at least 6 hours. The filtered extract was dried in a 60°C oven. The residue was dissolved in methylene chloride (1.0 ml for 25-mg residue) and a portion of this solution was spotted on a scribed and activated TLC plate coated with 250 μm alumina. After developing the spots in benzene-pentane (2:8), the BaP spot was scraped from the plate. The quantitative elution of BaP from the adsorbent was accomplished by shaking with pentane:acetone (95:5). The eluant was dried in a vacuum oven at 30 to 35°C and about 50 torr. The residue was dissolved in concentrated sulfuric acid and the fluorescence of this solution was recorded at excitation and emission wavelength of 470 nm and 540 nm, respectively. The concentration of BaP in the unknown was determined by comparison with standard BaP solutions.

For samples collected during the period 1973 to the present, the following modified procedure[174] was used for the quantification of BaP.

The quarterly composites of glass-fiber filter were Soxhlet extracted with cyclohexane for six hours. The extract was concentrated in a Kuderna-Danish evaporator at 50°C with a stream of dry nitrogen. 50 μl of the concentrate was spotted on a channelled 20% acetylated cellulose plate with a multispotter. Plates were developed with 2:1 ethanol:methylene chloride solvent mixture and air dried. The BaP spot on the plate was scanned with a thin layer plate scanning attachment at excitation and emission wavelength of 388 nm and 430 nm, respectively. The concentration was evaluated from the integrated strip chart area reading obtained by a digital integrator.

Since the BaP in this procedure did not separate from anthanthrene, the plate was then scanned at an excitation and emission wavelength of 434 nm and 470 nm, respectively, for the determination of anthanthrene concentration. The anthanthrene contribution in BaP could be corrected by subtracting 16% of anthanthrene concentration from the BaP reading (at BaP wavelengths, anthanthrene was found to be 84% less efficient).

4.7 DIFFICULTIES IN POM MONITORING AND ANALYSIS

The data reported on the levels of POM in different sources are susceptible to various kinds of errors and uncertainties at different stages of the monitoring and analytical phase. The first source of uncertainty in monitoring POM levels arise from the lack of knowledge regarding the degradation of these compounds under environmental conditions (see Section 3.3). The evaluation of adverse health effects requires a judicious selection of parameters that need to be monitored. The selection process becomes difficult in the absence of specific information regarding the form of POM that may exist in a certain atmosphere as a result of environmental reactions. Even in cases where it has been decided to monitor a specific form of POM, e.g., a selected group of PAH, the reported values are subject to substantial errors. Such errors and uncertainties may arise during defining the monitoring strategy, sampling procedure, transportation and storage of samples, and analytical procedures. Possible sources of error in each of the individual categories will be discussed in the following sections.

4.7.1 Sampling Errors

4.7.1.1 Nonisokinetic sampling--Most ambient sample collections are done non-isokinetically: That is, as the air containing the particulate matter is drawn into the sampler inlet, the speed and direction of the air are changed. The inertial characteristics of suspended particulates bring about losses of larger particles both by drift from the sampled air stream and by impaction on the surface of the sampler inlet. Although it is difficult to estimate the error arising from nonisokinetic sampling, such errors may be minimized by avoiding eddy current, turbulence, divergence or convergence, and changes in the direction of the sampled air stream.

4.7.1.2 Non-incorporation of sampling efficiency--The evaluation of sampling efficiency is particularly difficult under ambient atmospheric sampling conditions. The percent recovery of a collected sample is, however, rarely determined either during ambient or source sampling. The lack of incorporation of sampling efficiency lends considerable uncertainty in the reported POM levels.

4.7.1.3 Evaporative losses and reactive conversion--Collection of POM on widely-used glass-fiber filter alone without the back-up adsorbent is subject to two kinds of errors. First, lower-molecular-weight compounds on adsorbed particulate phase may have some equilibrium vapor concentrations under atmospheric conditions. This vapor part will escape collection on the filter. Second, air passing through a filter containing the collected substances will carry away amounts equal to or less than the equilibrium vapor concentration because of desorption from the adsorbed substrate. Quantitative data on losses of POM from soot particles collected on filter have been reported by Thomas et al.,[37] DeWeist and Rondia,[24] Murray et al.,[38] and Rondia.[39] Pupp et al.[40] determined the equilibrium vapor concentration for a number of POM and concluded that considerable losses occur during ambient sampling of compounds having an equilibrium vapor concentration of 0.5 x 10^{-3} mg/m^3 or higher at ambient temperature. Considerable losses are expected with pyrene, anthracene, phenanthrene and BaA. However, the equilibrium vapor concentration of compounds adsorbed to particulate matter may be quite different and lead to much different conclusions.

Besides sublimation, the chemical changes of POM which may occur on the sampling probe and on the filter paper require further investigations. The first of the chemical changes may occur as the result of catalytic effects of the probe material used during sampling. Hermann[42] studied this effect and concluded that neither Vycor nor stainless steel used as probe material causes any catalytic effect in promoting chemical reactions. However, there are many oxidants in the air which are drawn through the filter during sampling. Lane and Katz[43] using simulated atmospheric conditions have demonstrated that POM coated on a glass surface will be rapidly oxidized by the prevailing oxidants in the atmosphere. Their work also indicates that BaP is more prone to oxidation than BbF.

Since atmospheric POM exist on the surface and in the interstices of soot particles in a multi-layered form, the surface-exposed POM on the filter may be oxidized rapidly by the prevailing oxidants in the atmosphere followed by a slower penetration reaction of the subsurface POM and POM adsorbed in the pores of particles. However, reactions in the latter two cases can be expected to be slower not only because the oxidants have to penetrate to their surfaces but also because a layer of oxidized material may protect them from further oxidation. Quantitative data regarding this oxidative conversion of POM during collection are not available. There is also conflicting evidence regarding the importance of this process. For example, by passing clean air at a rate of 100 liters/min through filters containing airborne particulates Matsushita et al.[41] found negligible losses of PAH when air treatment was less than 7 days. But by passing air for 3 weeks at a rate of 3 liters/min through filters containing particulates, Commins[168] reported 76 percent and 87 percent losses of fluoranthene and pyrene, respectively. No significant losses were observed for high molecular weight compounds, including BaP. Sawicki pointed out that he failed to observe significant changes in pyrene concentrations under similar conditions.[168] Whether the lower temperature of the filter and the passed air was responsible for the lack of loss was not clear from his comments in the above reference. It is also not clear from these experiments whether the losses of PAH are due to oxidative conversion, purely evaporative processes or both.

4.7.2 Losses During Transportation and Storage

Another source of error that needs to be properly addressed is the loss of POM during transportation and possible storage of the collected samples prior to analysis. Losses during this period can occur from three sources: (1) photooxidation, (2) volatilization, and (3) chemical oxidation. The first of these losses can be easily prevented by transporting and storing the sample in the dark. Losses of POM due to the other two factors have been noted by various investigators. A decrease in the concentrations of fluoranthene, phenanthrene, and pyrene by 40 to 60 percent, and rearrangement of dibenzo[a,l]-pyrene to dibenzo[a,e]fluoranthene and BaA to chrysene have been noted by storing the sample for 3 weeks.[169] Hermann[42] has noted considerable error due to degradation or reaction when storing the sample at room temperature and exposing it to air. Similar results have been observed by other investigators; the results of Commins[168] are shown in Table 4-11.

Since the samples in Table 4-11 were stored in the dark at room temperature and were exposed to air, the losses may be due to oxidation, sublimation, or both. By storing the sample in glass bottles not exposed to air, Hermann[42] reported no significant loss of POM. The storage of samples at cooler temperature (in a refrigerator or in contact with ice) will further prevent the possibility of losses due to both volatilization and chemical reaction.

Table 4-11. PERCENTAGE LOSSES OF POM FROM FILTER PAPER DUE TO VARIATION OF STORAGE TIME[168]

Compound	% losses under different storage times[a]	
	3 wks. at room temp.	1 yr. at room temp.
Fluoranthene	37	92
Pyrene	34	88
Benzo[a]pyrene	5	32
Benzo[e]pyrene	+22[b]	23
Anthranthrene	+9[b]	21
Benzo[ghi]perylene	9	10
Coronene	-	1

[a] The samples were stored in sealed envelopes.
[b] These are the % gain (instead of loss) observed.

4.7.3 Errors In Analytical Procedures

4.7.3.1 Losses during desorption process--Several sources of loss are possible during analytical treatment of samples. The first of these takes place during desorption of POM from the sampling filter. The loss during this step depends on the nature of POM and the method used for desorption. The widely used method of Soxhlet extraction can give poor recovery for some POM and the recovery is dependent on the solvent used (see Section 4.3.1.1).

4.7.3.2 Column and thin-layer chromatographic and storage losses--Analytical procedures involving column chromatography can cause losses of POM either due to irreversible adsorption or photoreaction.[85] The photoreactivity of a number of POM in some adsorbed phase is considerably greater than in solution. Similar losses due to photoreaction on TLC plates have been observed by Inscoe.[170] Therefore, it is absolutely necessary that all analytical procedures be performed with long wavelength (red) light of low intensity. The storage of samples during analysis should, likewise, be done at -4°C and in the dark to avoid losses.

4.7.3.3 Losses in evaporative concentration step--The loss of POM in evaporative concentration steps is another most frequently encountered source of error. Fox and Staley[31] reported a recovery of only 81 to 83 percent of BaP when the flash evaporation of solvent from extract was allowed to proceed to dryness. The loss from this step can be expected to be even greater for other POM with higher volatility, such as pyrene, BaA, anthracene, and phenanthrene.

4.7.3.4 Errors due to incomplete resolution--The greatest discrepancies in the reported literature values for monitored POM (as in 1-Aza-fluoranthene in Table 5-5) probably arise due to incomplete resolution of isomeric compounds.

In some instances separations of isomers are very important from a toxicological point of view because two isomers may exhibit different carcinogenic characteristics, e.g., BaP and BeP. Some of the hard-to-separate isomeric compounds are: BaA, chrysene, and triphenylene; BbF and BkF; BaP and BeP; benzo[b]chrysene and indeno[1,2,3-cd]pyrene; benzo[ghi]perylene and anthranthrene; and the alkyl- substituted products of the respective group of compounds. Most packed columns, including the widely used Dexsil-300 GC, will not separate these compounds.[106] Although the separation is better with Dexsil-400 GC columns, it is by no means complete.[106] Columns packed with liquid nematic crystals have been claimed to accomplish much better resolution.[116,117] But column bleed at higher temperature has restricted the use of this packing material. Improved resolution using WCOT columns has been obtained. However, using a 50-m capillary column coated with OV-1, Lunde and Bjørseth failed to separate BbF from BkF.[124] Lee et al.[56] used an 11-m capillary column coated with SE-52 and showed that alkyl- substituted derivatives of many isomers could not be separated.

TLC can provide better resolution when the mixture is spotted on the plates coated with composite adsorbents and developed in two dimensions with appropriate solvent systems. The limitation of the TLC method lies in its inability to separate a mixture when a multitude of components are present. Even in cases where separations can be achieved, losses are encountered during the quantification procedure no matter whether solvent elution of the TLC spot or *in situ* scanning is used.

The commonly used modern technique of HPLC separation has the advantage of better resolution of the hard-to-separate components already mentioned.

The reverse-phase packing material, μ-Bondapak C_{18}, provides a good separation of alkyl-substituted components. In complex samples containing alkyl-substituted as well as unsubstituted parent PAH, the use of reversed-phase HPLC could yield misleading results. For example, 1,3,5-trimethyl naphthalene, 1-methyl phenanthrene, and fluoranthene elute simultaneously with CH_3CN-H_2O mobile phase even though these compounds consist of two, three, and four rings, respectively.[145]

It should be mentioned that in some instances correction for the losses during the analytical procedures have been attempted by the use of internal standard(s). However, when the quantification of a number of parameters are attempted, correction by the addition of one or two internal standards may not be adequate since the losses are not uniform and vary from compound to compound.

4.7.4 Uncertainty in the Number of Parameters to be Monitored

Lee et al.[56] compiled a list of more than 100 PAH compounds which have been detected in airborne particulate matter. The list could be considerably higher if other neutral and basic aza-arenes are included. The vastness of the POM and the uncertainty in the number of parameters which need monitoring constitutes one of the biggest problems. Certainly it is necessary to determine the levels of hitherto undetected compounds which may be present in the air in order to evaluate their potential health effects. But a cost-effective routine analysis requiring good precision and accuracy often demand that the number of parameters be restricted to a few selected ones. Therefore, there is a great need to define a few representative POM which will measure the carcinogenic potential of atmospheric POM to man.

4.8 ECONOMIC CONSIDERATION IN THE SELECTION OF THE ANALYTICAL TECHNIQUE

The cost of POM analysis will depend on the selected method of analysis. The method selection is, in turn, dependent on the objective of the analysis. When a method is intended to be used for dosimetric purposes in occupational settings, it must be capable of real-time monitoring of POM in the event of a process leak or other increase of the POM content in the working atmosphere. In addition to this requirement, integrating dosimeters are needed for determining the integrated exposure received by workers and the extent of contamination of the tools and the working areas. Irrespective of the economic considerations the commonly used analytical methods are time-consuming and do not meet these dosimetric requirements. Monitoring instruments, such as time-resolved fluorescence, correlation spectroscopy, second derivative spectrometers, and other methods which fulfill dosimetric requirements, are in the developing stages and it is difficult to project cost estimates for these methods. The reader is referred to a report by Hawthorne et al.[171] for a review on this subject.

On the other hand, several analytical methods are presently available for the determination of the nature and the level of POM collected from environmental samples. The selection of a particular analytical method depends on, (1) number of parameters to be determined, (2) desired accuracy for each determination, and (3) sensitivity of the analytical method which determines the lowest detection limit of the measured parameters. A comparative cost estimate, through-put, and other relevant data for four commonly used techniques is shown in Table 4-12. All the data in this table are estimates with some values taken from available literature.[31,172] These costs do not include the cost of initial survey, sample collection, method validation, etc., which can be a considerable cost factor in any sampling program.

Table 4-12. COMPARATIVE COST, THROUGHPUT, AND OTHER RELEVANT DATA FOR FOUR COMMONLY USED ANALYTICAL METHODS.

	Methods			
	TLC/fluor.	Column chrom/U.V.	GC-MS-Computer	HPLC-fluor.
Applicability	2 compounds	~12 compounds	80-125 compounds	10-15 compounds
Operational cost/sample	$11.25	~$600	$800	< $13
Sample turnaround	1 day	3 days	80 min.	65 min.
Samples/day	8	1/3	6	7
Sensitivity, analytical	1-2 ng	~1 μg	>10 pg	25-50 pg
Capital and peripheral investments, initial	~$12,800	~20,000	$150,350	~$16,000

It can be concluded from Table 4-12 that GC-MS-computer technique is preferred over other methods for non-routine analysis involving a vast number of parameters. The initial capital investment for this method is extremely high, although the recurring expenditure is comparatively low. For routine analysis of a dozen or so parameters, HPLC-fluorimetric technique appears most promising in view of its cost effectiveness. The TLC fluorimetric method should be the method of choice when one or two parameters are intended to be monitored. This method needs the least amount of capital investment and operator skill for performing the analysis.

It should be pointed out that Table 4-12 is not intended to include all the available methods, but rather serves as a guideline for the evaluation of cost effectiveness between methods. It is also evident that the selection of a particular analytical method is rarely based on pure economic considerations alone, but primarily depends on the intended objective of the analysis. The vast number of POM in the environment and the uncertainty in the number of parameters which need monitoring constitutes one of the biggest problems in the consideration of cost evaluation. Certainly, monitoring of BaP as an indicator for environmental POM pollution is cost effective; but the selection of this parameter alone for the aforementioned purpose remains questionable. One of the great needs in this regard is to define a few representative parameters (instead of BaP alone) which will be indicative of carcinogenic potential of environmental POM to man and restrict the monitoring to these parameters. This will be one of the most important steps towards cost reduction in POM analysis.

4.9 SUMMARY AND CONCLUSIONS

Polycyclic organic matter (POM) in environmental samples consist of a vast array of compounds. Some of these compounds show carcinogenic activity necessitating the need for their monitoring. The monitoring is usually performed with the objective of determining the particle size distribution and evaluating the nature and concentrations of a few individual components in the sample. This section briefly describes the various methods available for sampling POM from various sources and the analytical methods presently available for quantification of data from the collected samples. Particular emphasis has been placed on the difficulties commonly encountered in the various monitoring phases.

Collection of various source samples is usually performed by filtration through a suitable medium. With high temperature effluent samples, the air is passed through a cooling train prior to passage through the filtering medium. High-volume samplers are used commonly to collect total ambient particulate matter with glass-fiber filter (99 percent efficient for 0.3-μm particles). Some investigators have used additional back-up filters consisting of adsorptive media to collect POM present in the vapor phase and to prevent evaporative losses of POM. The high-volume air samplers do not provide information relative to aerosol particle size-weight distribution which is essential for the prediction of areas of deposition in man as a result of inhalation of aerosol. Cascade or cyclone impactors are often used for this purpose. Limited information is available on sample recovery from sample collection procedures. More research is needed to establish the extent of evaporative and oxidative losses which may occur during sample collection.

The recovery of POM from the widely used Soxhlet extraction for desorption of POM from the filtering/adsorbing medium is poor for some compounds and is dependent on the solvent(s) used. A method utilizing dissolution by sonification yields better results. Techniques which use sublimation and a thermal method for desorption of POM from collection medium are promising, although the latter method is only applicable when coupled with gas chromatographic analysis.

The problem of any analytical method for the quantification of POM, which usually occur as a small quantity in a large matrix of impurities, is three-fold. First, the compounds of interest must be adequately separated from impurities to the extent necessary for their interference-free detection. Second, the POM (many of which are isomeric compounds) must be adequately resolved from each other. Third, a high sensitivity of detection is required to quantitate small amounts of POM usually collected from environmental samples.

Separation procedures involving solvent partitioning is generally used for the isolation of POM from paraffinic hydrocarbons. Column chromatography with alumina, silica gel, Florisil, or, occasionally, cellulose, and cellulose acetate, has been used not only for the enrichment of POM but also for the resolution of the individual POM. However, separation is rarely complete and quantitative. Gel filtration produces better resolution but the procedure is very time consuming.

TLC is widely used for resolution of POM, particularly the isomeric compounds. The method is quick and inexpensive but is hardly suitable for separations involving a large number of compounds. Moreover, this method results in some losses of POM. Gas chromatography offers a good method for the separation of POM. The separation is more quantitative than either column or thin-layer chromatography. With the exception of liquid nematic crystal phases, most liquid phases used in GLC do not separate isomeric compounds. Even with nematic crystals, column bleed at higher temperatures has limited this packing material to lower temperature use. Better resolution is achieved with wall-coated open tubular capillary columns.

HPLC offers several advantages including higher speed, higher resolution, and lower operating temperature than gas chromatography. The method is non destructive and the injected sample can be recovered easily. This technique is gaining wide acceptance as a modern method for the separation of POM, although the separation of isomeric POM from real-life samples may not be complete.

With regard to detection, flame ionization and electron capture detectors are used when the mode of analysis is gas chromatographic. Fluorescence spectroscopy has, however, become well established as a sensitive and selective analytical technique for POM when other chromatographic procedures are used. Several groups have found it to be at least ten times more sensitive than the U.V. method. It is also more sensitive and less expensive than mass spectral detectors. Mass spectrometers normally have a nanogram detection limit, although integrated ion-current techniques reduce this limit to sub-picogram range.

No single analytical separation procedure to date is capable of providing complete separation and resolution of the POM fractions. Therefore, POM is actually analyzed with a combination of separation and detection methods. The selection of analytical methods is dependent on the number of parameters to be monitored and the required accuracy and cost of analysis. In the past, the most widely used combined method consisted of column chromatography followed by TLC and U.V./fluorescence detection. More recent methods utilize column chromatography in combination with HPLC and U.V./fluorescence detection. When the number of parameters to be monitored is very large, column chromatography coupled with gas chromatography-mass spectrometry is used as the method of analysis. In order to provide valid quantitative results, appropriate corrections for analytical recovery of the POM should be provided through the addition of internal standards.

The widely accepted practice of monitoring benzo[a]pyrene alone, as an indicator for other POM, is questionable. More research effort should be directed towards defining a few representative parameters which would be indicative of carcinogenic potential of POM present in the environment.

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5. AMBIENT LEVELS

5.1 AMBIENT ATMOSPHERIC LEVELS

A variety of polycyclic aromatic hydrocarbons (PAH), aza-arenes, and carbonyl-arenes have been detected in ambient air. Because of its carcinogenic properties, benzo[a]pyrene (BaP) has been the most extensively monitored and has frequently been used as an indicator of PAH. However, the relative amount of individual POM compounds released from different sources can vary considerably, but most are present in ambient air samples. For example, automobile emissions contain a relatively low amount of BaP as compared to other PAH. Also, there are several other social or occupational sources of POM which may result in larger exposures to humans than those from ambient air. For example, Bridbord et al.[1] have tabulated human exposure to PAH in terms of BaP from a number of occupational and other sources (Table 5-1). This treatment suggests that smoking one pack of cigarettes a day results in an appropriate exposure of BaP twenty times greater than that of a person exposed to ambient air BaP levels (assumed 2 ng/m^3 BaP). The exact amount of BaP intake is difficult to determine because of the different modes of inhalation during smoking, working, or exposure to ambient air.[2] Bridbord et al.[1] also concluded "that to the extent that polynuclear aromatic compounds besides BaP are also present, BaP represents a poor surrogate..."

Table 5-1. INFLUENCE OF OCCUPATIONAL AND OTHER FACTORS UPON BENZO[a]PYRENE EXPOSURE[1]

Factor	BaP Exposure (μg/day)	Cigarette equivalents (packs/day)	Source of data
Smoking one pack of cigarettes each day	0.4	1	NAS report 1972
Coke oven workers			
Top side workers	180	450	NIOSH data[a]
Side and bench exposure	70	175	NIOSH data[a]
Coal tar pitch worker	750	1875	1962 report
Airplane pilots			
Transatlantic flights	0.93	2.3	NIOSH data[a]
Domestic cross country	1.38	3.5	NIOSH data[a]
Employee in restaurant	0.8	2	1975 data
Person living near expressway 24-hr/day (adverse meteorology)	0.02	0.05	1976 projection from 1974 data
Commuter on an expressway 2-hr/day (adverse meteorology)	0.04	0.10	1976 projection from 1974 data
Expcsure to ambient BaP levels 8-hr/day	0.02	0.05	assumed exposure to 2 ng/m^3

[a] Unreferenced NIOSH data--presumably the latest data in 1976.

Concentrations of BaP are different in various cities and at different times of the year. In general, the concentrations are usually highest during the winter months (Figure 5-1), probably due to heating sources.[3,4] However, there are some exceptions. Cleveland, for instance, does not follow the high winter-low summer concentration pattern. It has been suggested that this may be due to significant industrial emissions of BaP that would be uniform throughout the year.[3] The higher BaP concentrations in the winter in most cities are not due to an increase in particulate matter, but rather can be attributed to an increase in the amount of BaP in the particulates.

From 1966 to 1970, the benzene-soluble organics (BSO) of quarterly composite samples of suspended particulate matter collected on glass-fiber filters (99 percent efficient for particles of 0.3 μm) by the 250 National Air Surveillance Network (NASN) stations were analyzed for BaP. These data, in the form of annual arithmetic averages for the numerous NASN urban and non-urban stations, are presented in Tables 5-2 and 5-3 for the years 1966 to 1970. The quarterly composite average for all the stations during the same years is presented in Figure 5-1. The levels from year to year remain relatively constant and do not necessarily correlate to city size.[5] Particularly interesting is Los Angeles, where high auto emissions do not result in high BaP levels. During the period from 1971 to 1976 a more limited number of NASN stations (40) were sampled for BaP. The data from these stations for the total period from 1966 to 1976 are presented in Table 5-4.

Other polycyclic organic compounds are frequently found in higher concentrations than BaP. Sawicki[4] found that benzo[ghi]perylene (BghiP) concentrations during the summers of 1958 to 1960 were higher than BaP levels in the

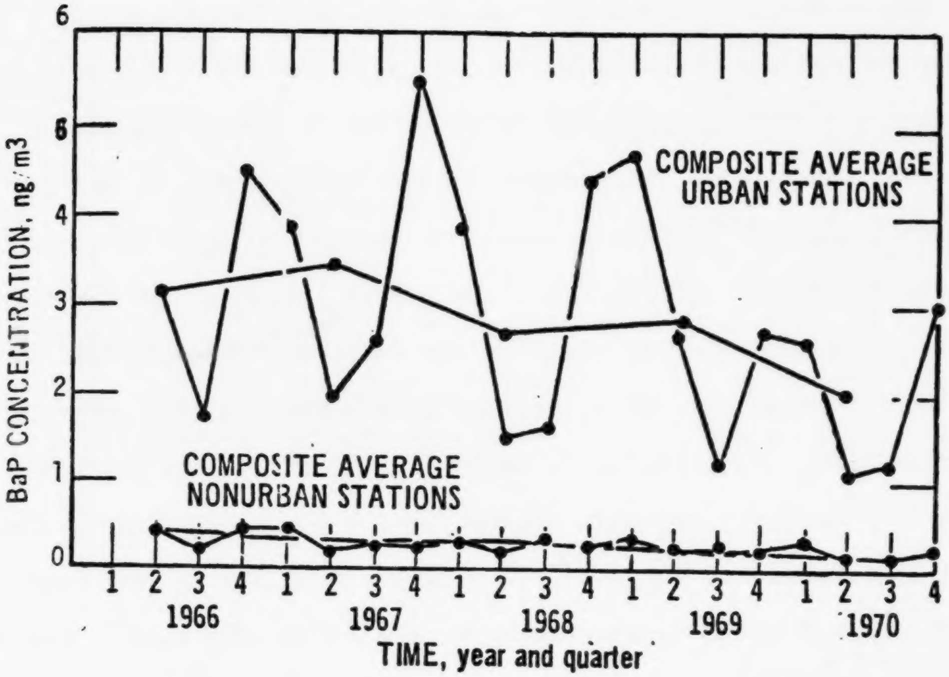

Figure 5-1. Composite quarterly averages for BaP at 32 urban and 19 nonurban NASN stations.[10]

Table 5-2. ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS AT NASN URBAN STATIONS[5] (ng/m^3)

Station	1966	1967	1968	1969	1970
Alabama					
Birmingham	18.5				
Gadsen	3.5		2.4	1.8	2.5
Huntsville		3.1	2.7	1.8	1.6
Mobile	6.5		4.2	2.6	
Montgomery		2.3	2.9	2.0	1.3
Alaska					
Anchorage	2.3	1.9	1.7	1.3	0.8
Arizona					
Phoenix	1.7	2.5	2.1	2.2	
Tucson	0.6	0.7	0.7	0.5	0.4
Arkansas					
Little Rock	1.2	0.9	0.9	1.1	0.7
West Memphis	1.1	2.2	2.2	2.4	0.6
California					
Burbank	2.5			2.9	1.9
Glendale		1.0	1.6	1.6	1.0
Long Beach		2.1	2.1	2.3	1.0
Los Angeles	2.1	1.3	1.8	1.9	1.2
Oakland	2.7	1.7	1.6	1.6	1.0
Ontario			0.9	0.6	0.6
Pasadena	1.8		2.3		0.7
Riverside			1.3	0.8	0.7
Sacramento			1.4	1.8	0.7
San Bernardino			1.0	0.9	0.8
San Diego	1.7	1.6	1.2	1.4	0.7
San Francisco	1.1	1.5	1.8	1.2	0.6
Colorado					
Denver	2.3	2.4	2.3	2.5	2.2
Connecticut					
Hartford	2.3	2.1	1.4	2.0	1.4
New Haven	3.5	1.9	1.4	2.1	1.2

Table 5-2 (Cont'd). ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS AT NASN URBAN STATIONS[5] (ng/m^3)

Station	1966	1967	1968	1969	1970
Delaware					
Newark	1.0	1.4	0.9		0.4
Wilmington	2.2	2.7	1.9	1.7	1.1
District of Columbia	2.4	1.9	1.9	4.3	
Florida					
Jacksonville			2.9	2.3	1.4
Tampa			1.5	1.0	0.5
Georgia					
Atlanta	1.4	3.0	1.8	1.9	0.9
Hawaii					
Honolulu	0.2	0.5	0.6	0.6	0.2
Idaho					
Boise City	3.5	2.4	2.0	6.0	1.1
Illinois					
Chicago	3.3	3.0	3.1	3.9	2.0
Springfield			1.1	1.3	0.9
Indiana					
East Chicago	6.8	5.7	1.9	6.8	5.3
Hammond	3.9	2.5	2.1	3.3	1.7
Indianapolis	10.4	5.7	4.1	5.2	2.3
Muncie	2.4	1.6			
New Albany	5.4			4.3	3.7
South Bend	2.2	2.6	3.7	3.7	2.4
Terre Haute		3.7		4.0	2.8
Iowa					
Davenport	3.2			1.7	0.9
Des Moines	2.5	2.7	1.1	0.9	0.7
Cedar Rapids		0.8	0.7		0.3
Kansas					
Kansas City	1.2		1.2	1.1	2.4
Topeka		0.5	0.7	0.4	0.3
Wichita	0.8	0.5	1.0	0.7	0.5

Table 5-2 (Cont'd). ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS AT NASN URBAN STATIONS[5] (ng/m^3)

Station	1966	1967	1968	1969	1970
Kentucky					
Ashland	10.5		9.3	10.9	6.7
Covington	3.1	1.9	3.6	4.1	4.4
Lexington		1.8	3.0		1.6
Louisville	2.5	2.1	2.7	1.9	
Louisiana					
New Orleans	2.3	1.8	1.6	1.5	1.1
Maine					
Portland			2.3		1.1
Maryland					
Baltimore	2.8	3.8	2.3	2.8	2.1
Massachusetts					
Worchester			1.7	1.5	1.6
Michigan					
Detroit	4.7	5.4	5.1	3.9	2.6
Flint		1.4	0.8	1.7	1.5
Grand Rapids		2.8	3.4	1.7	0.9
Trenton			1.4	1.6	0.8
Minnesota					
Duluth	2.2		2.7	2.1	1.1
Minneapolis	1.6	1.3	1.1	1.4	0.6
Moorhead	0.7		0.9	1.0	1.6
St. Paul	1.8	2.3	1.8	1.8	1.0
Missouri					
Kansas City			1.8	1.6	1.1
St. Louis		2.3		3.3	
Montana					
Helena		0.8	0.9	0.5	
Nebraska					
Omaha	2.7	1.3	1.9	1.6	1.0

Table 5-2 (Cont'd). ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS AT NASN URBAN STATIONS[5] (ng/m^3)

Station	1966	1967	1968	1969	1970
Nevada					
Las Vegas	1.3	1.1	1.4		
Reno		4.6	3.1		
New Hampshire					
Concord	0.6	1.5	1.0	0.7	0.6
New Jersey					
Camden	3.0		1.6	2.4	1.9
Glassboro	0.7	0.8	1.2	1.1	1.2
Jersey City	4.2	3.5	2.3	2.7	4.7
Marlton	1.2	1.6	1.3		1.4
Newark	2.1	3.3	2.1	1.8	1.5
Patterson		1.9	2.0	1.2	1.2
Perth Amboy	2.1	2.1	1.2	1.2	1.0
Trenton	2.2		1.0	1.5	1.1
New Mexico					
Albuquerque	2.0	1.9	1.8	1.1	1.1
New York					
New York City	4.1	3.9		3.6	3.0
North Carolina					
Charlotte	5.7	6.3	5.6	4.9	1.9
Durham			8.0	3.4	3.9
North Dakota					
Bismarck			0.9	1.0	0.4
Ohio					
Akron	4.1	3.7	3.0		
Cincinnati	3.6	1.9	1.8	2.9	2.6
Cleveland	3.1	2.9	3.0	3.8	2.8
Columbus	2.9	1.7	2.2	2.7	1.6
Dayton	2.7	3.7	2.4	1.9	1.5
Toledo	1.8	1.9	1.8	1.5	1.4
Youngstown	7.3	8.2	5.6	9.9	7.1

Table 5-2 (Cont'd). ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS AT NASN URBAN STATIONS[5] (ng/m^3)

Station	1966	1967	1968	1969	1970
Oklahoma					
Oklahoma City	1.5	0.7	0.7	0.7	0.9
Tulsa	0.7	0.6	0.8	0.5	0.8
Oregon					
Eugene		2.4			
Medford		4.8	8.2	4.1	
Portland	3.3	3.5	4.1	2.6	2.3
Pennsylvania					
Allentown		1.8	1.2	1.9	2.4
Altoona		29.5	18.0	22.3	19.3
Bethlehem		2.9	2.1	2.0	2.7
Harrisburg			1.3	1.5	1.5
Lancaster	2.3				
Philadelphia	3.8	5.9	2.9	4.0	2.4
Pittsburgh	4.9	7.0	6.3	13.8	5.9
Reading	2.3	2.9	2.4	1.8	1.6
Scranton		5.2	6.1	7.7	2.9
Warminster	0.9	2.2	0.9	1.0	
West Chester		1.1	1.0	1.3	
Wilkes Barre			1.6	1.5	1.3
York		1.8	1.9	2.0	1.2
Rhode Island					
East Providence		1.6	1.2	1.2	1.2
Providence	3.6	2.8	2.0	2.2	2.1
South Carolina					
Columbia		4.2	6.2	1.3	
Greenville	5.0		18.6	7.0	3.4
Tennessee					
Chattanooga	8.4	22.9	7.4	4.2	5.5
Knoxville		7.0	9.8	4.7	
Memphis	1.7	1.6	1.3	0.7	1.4
Nashville	5.5	7.0	6.0	2.8	3.6

Table 5-2 (Cont'd). ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS AT NASN URBAN STATIONS[5] (ng/m^3)

Station	1966	1967	1968	1969	1970
Texas					
Dallas	1.4			2.0	1.9
Houston	0.9				1.2
San Antonio	0.6	1.4	0.9	0.6	1.0
Utah					
Ogden	0.5		0.8	0.7	2.5
Salt Lake City	1.2	0.7	1.0	0.7	1.4
Vermont					
Burlington	0.8		0.7	0.5	0.7
Virginia					
Danville	3.2		2.5	1.8	2.7
Hampton		2.2	1.5	0.9	1.1
Lynchburg		9.2	8.7	6.3	4.5
Norfolk	2.8	3.5	4.9	3.9	1.8
Portsmouth		7.7	10.2	3.4	4.9
Richmond		5.2		2.2	2.1
Roanoke		7.5	7.7	5.3	6.2
Washington					
Seattle	2.7	1.8	2.0	1.6	1.5
West Virginia					
Charleston	3.4		4.6	2.6	2.1
Wisconsin					
Kenosha			1.4	1.7	1.3
Madison			1.3		1.1
Milwaukee	4.1		4.7	4.0	2.5
Superior			3.3	1.6	1.5
Wyoming					
Casper			0.9	0.6	0.4
Cheyenne	0.5		0.6	0.5	0.4

Table 5-3. ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS
AT NASN NONURBAN STATIONS[5]
(ng/m^3)

Station	1966	1967	1968	1969	1970
Arizona					
Grand Canyon	0.3	0.2	0.2	0.2	0.1
Maricopa County		0.2	0.5	0.3	0.3
Arkansas					
Montgomery County	0.3	0.1	0.2	0.2	0.1
California					
Humboldt Coutny	0.4	0.4	0.3	0.5	0.1
Idaho					
Butte County			0.2	0.1	0.1
Indiana					
Monroe County	0.5		0.5	0.3	0.2
Parke County	0.9		0.4	0.3	0.4
Maine					
Acadia National Park	0.2		0.3	0.1	0.2
Missouri					
Shannon County		0.2	0.2	0.2	0.2
Montana					
Glacier National Park		0.3	0.4	0.4	
Nebraska					
Thomas County	0.2		0.2	0.1	0.1
Nevada					
White Pine County	0.1		0.1	0.1	0.1
New Hampshire					
Coos County	0.2	0.2	0.2	0.1	0.1
New York					
Jefferson County	0.2		0.2	0.3	0.2

Table 5-3 (cont'd). ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS AT NASN NONURBAN STATIONS[5]
(ng/m^3)

Station	1966	1967	1968	1969	1970
North Carolina Cape Hatteras	0.2		0.2	0.1	0.2
Oklahoma Cherokee County	0.2	0.2	0.2	0.2	0.2
Oregon Curry County	0.1	1.1	0.1	0.1	0.1
Pennsylvania Clarion County	1.5	2.1	1.0	1.2	1.2
Texas Matagorda County	0.3	0.1	0.2	0.1	0.3
Vermont Orange County	0.9		0.3	0.3	0.2
Virginia Shenandoah National Park	0.9	0.3	0.3	0.3	0.2

Table 5-4. ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS AT NASN URBAN STATIONS IN ng/m^3.[6]

	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976
Birmingham, Ala.	18.5	--	--	--	--	4.0	2.3	1.5	2.5	0.9	1.6
Gadsden, Ala.[a,b]	3.5	--	2.4	1.8	2.5	1.2	1.2	0.8	0.5	0.6	0.6
Montgomery, Ala.[a]	--	2.3	2.9	2.0	1.3	0.5	0.5	0.3	0.4	0.3	0.3
Grand Canyon Nat. Park, Ariz.	0.3	0.2	0.2	0.2	0.1	--	--	< 0.1	< 0.1	--	< 0.1
Jacksonville, Fla.[a]	--	--	2.9	2.3	1.4	2.2	0.4	0.2	--	0.4	0.4
Honolulu, Hawaii[a]	0.2	0.5	0.6	0.6	0.2	0.2	0.1	< 0.1	< 0.1	--	< 0.1
Chicago, Ill.[a,b]	3.3	3.0	3.1	3.9	2.0	2.5	1.3	0.4	--	1.0	--
Gary, Ind.[a,b]	--	--	--	--	--	1.6	1.2	0.3	0.5	--	0.7
Hammond, Ind.[a,b]	3.9	2.5	2.1	3.3	1.7	3.8	1.4	0.2	0.4	0.7	0.5
Indianapolis, Ind.[a,b]	10.4	5.7	4.1	5.2	2.3	0.9	4.9	0.4	--	--	0.6
Terre Haute, Ind.[a,b]	--	3.7	--	4.0	2.8	--	1.1	--	0.3	0.6	0.6
Ashland, Ky.[a,b]	10.5	--	9.3	10.9	6.7	9.0	8.5	2.9	--	4.7	2.9
New Orleans, La.[a]	2.3	1.8	1.6	1.5	1.1	0.9	0.4	0.3	0.3	0.2	0.2
Baton Rouge, La.[a]	--	--	--	--	--	0.4	0.2	0.1	0.1	0.1	0.3
Arcadia Nat. Park, Maine	0.2	--	0.3	0.1	0.2	--	0.3	--	0.1	0.1	0.1
Baltimore, Md.[a,b]	2.8	3.8	2.3	2.8	2.1	2.8	1.3	0.4	0.5	0.6	0.5
Detroit, Mich.[a,b]	4.7	5.4	5.1	3.9	2.6	1.4	1.9	1.0	--	1.0	1.1
Dearborn, Mich.[a,b]	--	--	--	--	--	--	0.6	1.0	1.7	--	--
Trenton, Mich.[a,b]	--	--	1.4	1.6	0.8	0.7	0.5	0.1	--	--	0.3
Duluth, Minn.	2.2	--	2.7	2.1	1.1	4.8	19.1	0.3	0.2	--	0.2
St. Paul, Minn.[a,b]	1.8	2.3	1.8	1.8	1.0	0.5	0.5	0.1	0.5	--	0.5
St. Louis, Missouri[a,b]	--	2.3	--	3.3	--	0.8	0.6	0.2	0.3	0.3	0.3
New York City, N.Y.[a]	4.1	3.9	--	3.6	3.0	2.3	1.8	0.7	0.9	0.9	0.5
Buffalo, N.Y.[a,b]	--	--	--	--	--	--	1.5	0.6	0.8	0.5	0.3
Cleveland, Ohio[a,b]	3.1	2.9	3.0	3.8	2.8	--	1.3	--	--	--	0.6
Toledo, Ohio[a,b]	1.8	1.9	1.8	1.5	1.4	0.8	0.4	0.4	0.2	0.4	0.5
Youngstown, Ohio[a,b]	7.3	8.2	5.6	9.9	7.1	3.7	3.2	1.1	1.9	2.1	1.5
Bethlehem, Pa.[a,b]	--	2.9	2.1	2.0	2.7	0.9	0.8	0.5	0.1	--	0.3
Erie, Pa.[a,b]	--	--	--	--	--	1.5	2.4	0.7	0.6	0.4	0.2
Philadelphia, Pa.[a,b]	3.8	5.9	2.9	4.0	2.4	2.3	0.9	0.6	0.8	0.6	1.0
Pittsburgh, Pa.[a,b]	4.9	7.0	6.3	13.8	5.9	6.1	10.6	--	1.3	2.1	2.0

Table 5-4 (Cont'd). ANNUAL AVERAGE AMBIENT BENZO[a]PYRENE CONCENTRATIONS AT NASN URBAN STATIONS IN ng/m^3.[6]

	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976
Chattanooga, Tenn.[a,b]	8.4	22.9	7.4	4.2	5.5	--	9.9	--	--	0.8	0.7
Houston, Texas[a,b]	0.9	--	--	--	1.2	0.5	0.4	0.4	0.2	0.2	0.1
Newport News, Va.	--	--	--	--	--	0.4	0.3	0.2	--	--	--
Norfolk, Va.[a]	2.8	3.5	4.9	3.9	1.8	1.2	0.6	0.4	0.2	0.2	0.5
Shennandoah Nat. Park, Va.	0.9	0.3	0.3	0.3	0.2	--	0.1	0.1	--	--	--
Seattle, Wash.[a]	2.7	1.8	2.0	1.6	1.5	0.5	0.5	0.3	--	--	--
Spokane, Wash.[a,b]	--	--	--	--	--	1.7	1.5	0.4	--	--	--
Charleston, W.V.[a]	3.4	--	4.6	2.6	2.1	0.9	0.7	0.2	0.5	0.5	0.4
Milwaukee, Wisc.[a,b]	4.1	--	4.7	4.0	2.5	1.8	3.6	0.6	--	1.1	0.4

[a] Urban site used for Figure 5-2.

[b] Urban site where a coke oven is located.

10 cities examined, but the reverse was true during winters in those communities that burn coal.

An early study of the various PAH compounds in urban air was reported by Sawicki and coworkers.[7] They analyzed eight compounds in seven cities during the summer of 1958 and winter of 1959. They collected their samples on glass-fiber filters, Soxhlet extracted with benzene, separated isomers with column chromatography, and quantitated the compounds from ultraviolet absorption spectra. Their results are presented in Table 5-5. Good relative correlation between BaP and the other PAH, except coronene, was found; the authors concluded that concentrations of the other compounds could be predicted from BaP data. The advantage of this is that costly and laborious work could be avoided if one can calculate other PAH values from BaP values. Hauser and coworkers, in an unpublished study of PAH in Birmingham, Ala.,[5] found a similar correlation between many of the compounds they examined (Table 5-6). Their results indicated the effect of localized sources on the immediate surroundings.

This correlation between the concentration of BaP and other compounds does not always occur. For example, a study by Kertész-Sáringer and Morlin[8] found little or no relationship between BaP and other PAH. They analyzed 11 PAH in Budapest air by collection on glass-fiber filters, Soxhlet extraction with benzene, and thin layer chromatography separation. Bands of the chromatographic plate were selected so that no more than three compounds were found in each band to be analyzed spectrophotometrically. The lowest correlation coefficient was found between BaP and chrysene or phenanthrene. Some relationship was found between pyrene and BaP, but the authors concluded that

Table 5-5. POLYCYCLIC AROMATIC COMPOUNDS IN THE AIR OF SELECTED CITIES[5]
(ng/m^3)

City	BghiP	BaP	BeP	BkF	P	Cor	Per	A	Total
Winter 1959									
Atlanta	8.9	7.4	4.7	6.0	6.0	4.3	1.1	0.5	38.9
Birmingham	18.0	25.0	10.0	13.0	17.0	3.5	5.5	2.2	94.2
Detroit	33.0	31.0	23.0	20.0	36.0	6.4	6.0	2.0	146.4
Los Angeles	18.0	5.3	8.1	5.7	6.0	12.0	1.6	0.2	56.9
Nashville	17.0	25.0	14.0	15.0	30.0	4.6	4.4	1.8	111.8
New Orleans	7.3	4.1	6.4	3.9	2.3	27.0	0.8	0.1	27.6
San Francisco	7.5	2.3	2.9	1.7	1.9	4.9	0.3	0.1	21.6
Summer 1958									
Atlanta	5.1	1.6	1.5	1.3	0.7	2.5	0.4	0.2	13.3
Birmingham	8.3	6.4	5.9	4.6	2.1	2.4	2.1	0.3	32.1
Detroit	9.5	6.0	5.3	4.9	2.8	1.8	1.7	0.4	32.4
Los Angeles	2.3	0.5	0.6	0.5	0.3	2.2	0.03	0.0	6.4
Nashville	3.4	1.4	1.2	1.0	0.6	1.3	0.2	0.1	9.2
New Orleans	4.6	2.0	3.1	1.8	0.3	2.5	0.4	0.1	14.8
San Francisco	2.6	0.3	0.5	0.2	0.1	1.6	<0.1	0.02	5.4

Table 5-6. ANNUAL AVERAGE CONCENTRATION OF PAH COMPOUNDS IN THE AIR OVER GREATER BIRMINGHAM, ALABAMA, 1964 and 1965

(ng/m^3)

City	Site	Fluor	P	BaA	Ch	BeP	BaP	Per	BghiP	A	Cor
Bessemer	1	7.0	7.6	7.8	13.1	10.5	13.5	1.3	14.1	1.2	2.6
Birmingham	3	4.9	4.6	5.3	8.1	7.6	9.0	0.9	9.5	0.7	2.7
	4	11.2	10.8	21.2	27.9	26.1	35.8	4.1	22.4	2.2	3.8
	5	10.8	9.1	14.5	14.2	15.0	20.5	2.0	15.3	1.2	3.5
	7	2.6	2.5	3.4	4.4	5.6	6.0	0.4	7.9	0.3	2.7
Fairfield	1	10.0	8.1	13.3	11.3	13.3	18.2	1.4	11.8	1.1	2.1
Irondale	1	3.4	2.8	4.2	5.7	6.3	7.6	0.6	7.0	0.4	1.9
Mt. Brook	1	1.0	1.0	1.0	2.2	3.0	2.6	0.2	3.7	0.1	1.4
Tarrant	1	3.4	3.6	3.9	7.6	7.6	7.4	0.8	8.2	0.2	2.2
Vestavia	1	1.0	1.0	1.0	2.0	2.9	2.4	0.2	3.5	0.1	1.2
Average		5.5	5.1	7.6	9.6	9.8	12.3	1.2	10.3	0.8	2.4

"the concentration of 3,4-benzpyrene[BaP] is a poor indicator for other compounds."

Gordon and Bryan[9] found similar results while monitoring PAH at four sites in Los Angeles. They found that the amount of coronene paralleled the estimated traffic density at the four sites. At three of the four sites, the PAH patterns normalized to coronene were similar and resembled patterns of auto exhaust. However, at the remaining site (near petroleum refineries and chemical plants), the PAH pattern, including BaP, was distinctly different.

An interesting monitoring trend has been developed from the NASN BaP values for the past ten years.[3,10,11] The declining trend for BaP during 1966 to 1970 is depicted in Figure 5-1 and is based upon BaP data at 32 urban and 19 non-urban stations. As can be seen, the average BaP concentration decreased from 3.2 nanograms/m^3 in 1966 to 2.1 nanograms/m^3 in 1970, approximately a 30 percent decrease.[10] A recent analysis[11] of BaP trends during the ten year period of 1966 to 1975 has been based upon 34 urban (24 with coke ovens) and 3 rural sites. These trends for the urban sites (Figure 5-2) are consistent with previous results indicating a steady decline of BaP. Even concentrations in the three remote rural areas (Grand Canyon National Park, Arizona; Acadia National Park, Maine; and Shennandoah National Park, Virginia) (Table 5-4) indicated a downward trend. The following limitations to the study were noted: (1) small number of areas were covered and data was only available from one single monitoring site (located in center city business area); and (2) a relatively high number of urban sampling sites were located in areas where coke ovens were present.

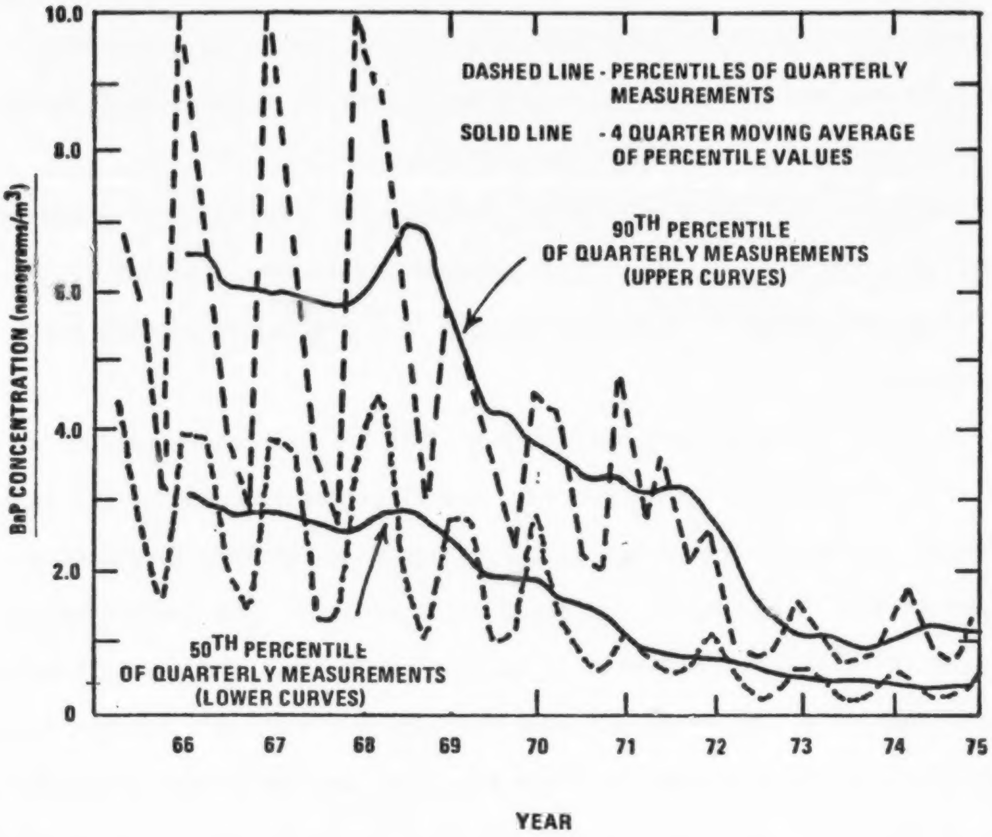

Figure 5-2 Benzo[a]pyrene - seasonality and trends (1966 to 1975) in the 50th and 90th percentiles for 34 NASN urban sites[11]

Although a large number of the NASN sites were in areas that had coke oven emissions, the monitoring stations were not sited for those emissions. Comparison of the coke oven sites to the non-coke oven sites showed that the coke oven sites had generally higher (40 to 70 percent) BaP concentrations and the downward trend for both types of sites was parallel. The authors concluded that the higher values could not necessarily be attributed to coke oven emissions alone since coke oven sites were usually located in more industrialized northern cities where industrial and heating sources may contribute significant amounts of BaP. Also noted was a decline of BaP summer levels for coke sites that was greater than the non-coke sites.

Examination of Figure 5-2 also illustrates the high winter-low summer seasonal concentration of BaP. "This seasonality is most dominant in the first five years (1966 to 1970) and is much less pronounced for the most recent years."

During the ten year period of 1966 to 1975 the BaP concentrations at urban sites decreased about 84 percent, dropping from a median of the individual annual average concentration of 3.2 nanograms/m^3 in 1966 to 0.5 nanograms/m^3 in 1975 (Fig. 5-2). The urban sites used for this trend analysis are indicated in Table 5-4 along with an indication of areas where coke ovens are located. Nashville, Tennessee was used by Faoro and Manning[11] for the trend analysis but is not presented in Table 5-4. The decline in BaP is believed to be due primarily to decreases in coal consumed in family dwellings or buildings in and around the central business districts of urban areas.[11] Also contributing to the decline is the improved disposal of solid wastes and the restrictions on open burning.[11]

Because of the importance of the NASN BaP monitoring data to the assessment of exposure, evaluation of the analytical procedure used is necessary. During the total period of 1966 to 1976 all the samples were collected on glass-fiber filter (99 percent efficient at 0.3 μm) with high-volume samplers. Twenty-four-hour samples were taken biweekly and sent to a central laboratory where individual samples were combined by quarter to save time and expense.[3] Up until 1972, the sample was extracted with benzene in a Soxhlet extractor for six hours followed by thin layer chromatography (TLC). BaP was removed from the TLC plate, dissolved in sulfuric acid, and measured by fluorescence spectroscopy. Starting in approximately 1973, a faster method of analysis was used which may have had some affect on the data generated. This consisted of cyclohexane extraction of the filter (cyclohexane was found to be as efficient in extraction as benzene), TLC separation, and fluorescence spectroscopy of BaP on the plate. Anthanthrene interferes with the latter procedure but its contribution to the fluorescence spectra can be subtracted using a characteristic emission band for anthanthrene.

The efficiency of collection and stability of BaP during the analytical procedure has not been completely examined. Many studies have indicated that BaP is primarily associated with particulate matter,[12,13,14] with a sizeable percentage (up to 60 percent) in the less-than-one-μm range.[15,16] How much BaP might pass through the 0.3-μm glass-fiber filter is unknown although the efficiency of collection should increase as the filter is loaded with particles. Also, DeWiest and Rondia[17] have provided experimental evidence that sizeable differences between the BaP collected by high volume samplers in summer as compared to winter temperatures will occur.

Stability during collection has been addressed by Commins.[18] He collected two identical samples of smoke on glass-fiber filters at 1.2 m^3 per minute for two hours. He then drew filtered air through one of the filters for two hours at the same flow rate. None of the eight hydrocarbons (including BaP) had changed significantly. A similar study by Sawicki[18] indicated no loss of BaP between identical samples whose only difference was that one set had twice as much air drawn through the filter. Thus BaP adsorbed on particulate matter that is collected on a glass-fiber filter appears to be stable to an identical volume of air. However, if oxidation of the outer layer of BaP occurs during the first sampling period but not during the second period because of protection of the inner BaP by the oxidized outer layer,[19] then the values for the above experiments should be the same. This possibility would result in lower reported BaP values than the quantities being inhaled.

Commins[18] also examined the stability of BaP on storage of filter paper or in cyclohexane. BaP from a smoke sample stored on a glass-fiber filter in a sealed envelope for a year lost 32 percent compared to a sample immediately analyzed. However, with BaP kept in cyclohexane in the dark for six months no loss was detected. Commins[18] concluded that the filters should be extracted as soon as possible. Since BaP samples are combined into three month samples and at times during the early 1970's backlogs of over a year were encountered, substantial losses of BaP could occur if the samples were not extracted soon after collection.

Declining trends for PAH have also been noted in Great Britian. Leahey[20] found that the concentration of BaP and BeP in downtown London was decreasing while coronene was remaining about the same. The author attributed the reduction of BaP and BeP to reduction in coal use and suggested that coronene had remained constant because of its greater proportion in auto exhaust which is increasing. The samples were collected on glass fiber filters, sealed in glass tubes at the end of each month, and analyzed at the end of a year. The filters were extracted with cyclohexane, separated by column chromatography (alumina), and analyzed by ultraviolet spectrophotometry.

Aza-arene compounds have recently received considerable monitoring effort. Brocco et al.[21] and Dong and coworkers[22] have detected a number of new compounds including quinolines, azafluoranthene, azapyrene, and isoquinolines. Their results are included in the tabulation in Table 5-8. The concentrations of aza-arenes are usually considerably lower than PAH, and the New York City monitoring of Dong et al.[22] are compatible with previous data. However, the results of the Brocco et al.[21] analysis of Rome air samples are considerably higher than any previously reported values. Many of these compounds (e.g., the quinolines) are very volatile and, therefore, the different values may be due to the different collection and analytical procedures which were used. Both Dong et al.[22] and Brocco et al.[21] collected their samples on glass fiber filters and then extracted with benzene[21] or benzene/methanol[22] (4:1). Brocco et al.[21] cleaned up their sample by TLC and analyzed by gas chromatography. Dong et al.[22] partitioned the aza-arenes into sulfuric acid, neutralized and extracted them back into chloroform, and analyzed by HPLC. Qualitative confirmation was accomplished by GC/MS.

Very few studies have examined the possibility of long term transport of POM which might result in POM contamination of areas that are downwind from large emission sources. Lunde and Bjørseth[26] monitored air samples in Norway which had different trajectories. They identified 20 different PAH (see Table 5-7) and determined that samples with trajectories from western Europe contained about 20 times more PAH than samples with trajectories from northern Norway or stationary air from southern Norway. When the wind was blowing from the right direction, the POM concentrations in Norway were of the same order of magnitude as in downtown London. This study is particularly important because it provides a list of at least 20 PAH compounds that are stable enough to be transported from the source to humans where they will be inhaled. At least four compounds (benzo[c]phenanthrene, benz[a]anthracene, benzo[a]pyrene, and indeno[1,2,3-cd]pyrene) which have well-resolved peaks are suspected carcinogens.

Perhaps the most detailed recent study of PAH compounds in atmospheric samples was conducted by Katz et al.[37] They analyzed ten individual PAH at five different sites in Ontario during three month periods in 1975 and 1976. Table 5-8 tabulates the results from two sites along with total PAH and the ratio of PAH and the BaP concentration. These results demonstrate a varying relationship between BaP and total PAH compounds.

Table 5-9 tabulates the various compounds for which monitoring concentrations have been reported. As many as 100 compounds have been found in urban atmospheres,[3,23] but they are not listed in Table 5-9 because quantitative information could not be found.

Table 5-9 illustrates the vast number of POM that have been detected in the atmosphere and there are probably many others that have not been quantitated for lack of an appropriate analytical method. The accuracy of the concentrations reported in Table 5-9 probably varies considerably, especially for the lower molecular weight compounds which are likely to sustain substantial losses by volatilization during collection on glass-fiber filter in high-volume samplers.

Table 5-7. CONCENTRATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN NORWAY AEROSOLS, ng/m^3.[26]

Sample Number	1	2	3	4
Sampling period	Feb. 20-21 1976	Nov. 25-26 1975	Jan. 25-27 1976	Feb. 1 1976
	Origin of air			
PAH	England, France	Northern Eng. Scotland	Northern Norway	Stationary air Southern Norway
Phenanthrene	4.725	1.216	0.036	0.146
Anthracene		0.278	0.038	--
Methyl-phenanthrene/-anthracene	0.661	0.216	--	0.052
Fluoranthene	6.637	3.965	0.171	0.324
Dihydrobenzo[a&b]fluorenes	0.874	0.363	0.032	0.032
Pyrene	4.864	3.293	0.135	0.286
Benzo[a]fluorene	0.815	0.318	0.021	0.026
Benzo[b]fluorene	0.571	0.149	0.117	0.148
1-Methylpyrene	0.147	0.099	0.009	0.009
Benzo[c]phenanthrene	1.021	0.957	0.038	0.108
Benz[a]anthracene	0.585	0.740	0.041	0.073
Chrysene/Triphenylene	1.756	3.269	0.099	0.194
Benzo[b&k]fluoranthene	4.312	4.013	0.083	0.464
Benzo[e]pyrene	1.191	2.635	0.066	0.135
Benzo[a]pyrene	0.965	2.053	0.059	0.098
Perylene	0.090	0.191	trace	0.011
Indeno[1,2,3-cd]pyrene	1.306	1.920	0.062	0.144
Benzo[ghi]perylene	1.142	1.971	0.064	0.140
Anthanthrene	0.225	0.423	0.007	0.022
Coronene	0.212	0.183	--	0.020
Total Identified PAH	32.099	28.252	1.108	2.435

Table 5-8. CONCENTRATION OF TEN POLYCYCLIC AROMATIC HYDROCARBONS IN ONTARIO CITY AIR, ng/1000m^3[37]

Comparative seasonal concentration levels of PAH's in air of Ontario cities April 1975-March 1976
[Location: Hamilton; site no. 29025]

	Apr.-June 1975	Jul.-Sept. 1975	Oct.-Dec. 1975	Jan.-Mar. 1976
Benzo(a)pyrene	1404	2351	3498	1934
Benzo(e)pyrene	606	1407	3771	1607
Benzo(b)fluoranthene	813	2626	7841	2297
Benzo(k)fluoranthene	419	1425	5145	443
Perylene	141	283	403	347
Dibenz(def,mno)chrysene	70	128	369	150
Benzo(ghi)perylene	5809	7183	7532	6418
Naptho(1,2,3,4def)chrysene	184	1017	2027	903
Benzo(rst)pentaphene	74	247	434	281
Dibenzo(b,def)chrysene	331	915	1132	704
Total	9851	17582	32152	15084
Divided by BaP	9.02	7.48	9.19	7.08

Comparative seasonal concentration levels of PAH's in air of Ontario cities April 1975-March 1976
[Location: Southern Sarnia; site no. 14061]

	Apr.-June 1975	Jul.-Sept. 1975	Oct.-Dec. 1975	Jan.-Mar. 1976
Benzo(a)pyrene	338	114	596	190
Benzo(e)pyrene	118	52	603	64
Benzo(b)fluoranthene	371	243	938	289
Benzo(k)fluoranthene	81	70	439	104
Perylene	27	13	87	19
Dibenz(def,mno)chrysene	23	8	44	7
Benzo(ghi)perylene	1038	1049	2700	1158
Naptho(1,2,3,4def)chrysene	828	61	434	129
Benzo(rst)pentaphene	422	15	69	23
Dibenzo(b,def)chrysene	508	81	213	107
Total	3749	1706	6123	2090
Divided by BaP	11.09	14.96	10.27	11.00

Table 5-9. POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, µg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Naphthalene	0.052-0.350	Providence area	1977	32
Anthracene	>1.9	Near industry handling coal, tar, pitch	1962	4
	0.1-2.2	Winter - seven cities monthly composite	1962	5
	0.0-0.4	Summer - seven cities monthly composite	1962	4
	0.068-0.278	Norway - air from N. England	1977	26
	6.15	Budapest	1975	8
Benz[a]anthracene	0.4-21.6	3 sites in Detroit (20 samples)	1965	24
	0.1-16.0	4 sites in New York (47 samples)	1966	24
	~4	Average U.S. urban atmosphere	1965	24
	0.74-0.041	Norway - air from N. England	1977	26
	11-14	Average urban air - Detroit	1965	27
	2	Average suburban air - Detroit	1965	27
	1.2-8.0	Rome	1975	28
	4.6	College Park, Md.	1976	29
	102	Baltimore Harbor Tunnel	1976	29
	8.2	Budapest	1975	8
	0.5-2.8	Los Angeles	1971	30
	0.18	Los Angeles	1976	31
	0.011 µg/g particulate	Summer - Toronto	1975	16
	0.022 µg/g particulate	Winter - Toronto	1975	16
	1.1-3.1	5 samples - Rome, Italy	1966	25
	0.1-13.1	14 samples - Rome, Italy	1972	25

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, μg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Dibenz[a,c]anthracene	4.5-0.029	Providence area	1977	33
Methyl-phenanthrene/anthracene	0.216-0.661	Norway - air from N. England	1977	26
Phenanthrene	>7.5	Near industry handling coal, tar, pitch	1962	4
	0.036-1.2	Norway - air from N. England	1977	26
	2.9-25	Rome	1975	28
	1.0	Budapest	1975	8
	0.011-0.340	Providence area	1977	33
Benzo[c]phenanthrene	1.021-0.038	Norway - air from N. England	1977	26
	0.9	Rome	1975	28
Benzo[a]fluorene	0.815-0.021	Norway - air from N. England	1977	26
Benzo[b]fluorene	0.571-0.117	Norway - air from N. England	1977	26
	1.1	Rome	1975	28
Dihydrobenzo[a&b]fluorenes	0.874-0.032	Norway - air from N. England	1977	26
Fluoranthene	0.19-15.0	3 sites in Detroit (6 samples)	1965	24
	∿4	Average U.S. urban atmosphere	1963	24
	6.6-0.17	Norway - air from N. England	1977	26

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, μg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Fluoranthene (Cont'd)	3.1-11.0	Rome	1975	28
	4.1	College Park, Md.	1976	29
	93	Baltimore Harbor Tunnel	1976	29
	1.04	Budapest	1975	8
	0.31	Los Angeles	1976	31
	1.5-0.16	Providence area	1977	33
	0.3-10.6	20 sites - Ontario (5 samples each location)	1966	25
	0.6-41	20 sites - Ontario (1962 inversion period)	1966	25
	0.06-2.6	1 site - Sidney, Australia (12 samples)	1965	25
	1.0-18.0	14 samples - Rome	1972	25
	2.1-4.5	5 samples - Rome	1966	25
	0.1-3.4	4 sites - Los Angeles	1973	25
Benzo[b]fluoranthene	2.3-7.4	3 sites in Detroit (6 samples)	1965	24
	0.54	Los Angeles	1976	31
	0.1-1.6	4 sites - Los Angeles	1973	9
Benzo[j]fluoranthene	0.8-4.4	3 sites in Detroit (6 samples)	1965	24
	0.17	Los Angeles	1976	31
	0.01-0.8	4 sites - Los Angeles	1973	9
Benzo[k]fluoranthene	1.1-15.7	2 sites in Detroit (6 samples)	1965	24
	1.7-20	7 cities, winter, monthly composites	1962	5
	0.2-4.9	7 cities, summer, monthly composites	1962	5
	0.2	Los Angeles	1976	31
	0.12-0.96	Toronto	1975	16

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, μg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Benzo[k]fluoranthene (Cont'd)	1.3-14.0	20 sites in Ontario	1966	25
	0.03-1.3	4 sites in Los Angeles (4 samples)	1973	9
Benzo[b&k]fluoranthene	4.312-0.083	Norway - air from N. England	1977	22
Benzo[ghi]fluoranthene	0.9-9.1	Rome	1975	28
Pyrene	1.3-19.3	3 sites in Detroit (6 samples)	1965	24
	Trace-35	12 U.S. cities	1962	24
	10	Near industry handling coal, tar, pitch	1962	4
	4.86-0.135	Norway - air from N. England	1977	26
	2.2-6.8	Rome	1975	28
	5.2	College Park, Md.	1976	29
	120	Baltimore Harbor Tunnel	1976	29
	2.06	Budapest	1975	8
	0.45	Los Angeles	1976	31
	0.18-3.8	4 sites in Los Angeles	1973	9
	0.08-4.0	1 site in Sidney, Australia	1965	25
	0.4-12.0	20 sites (5 samples at each site) in Ontario	1966	25
	0.4-17.0	14 samples - Rome	1972	25
	2.4-5.1	5 samples - Rome	1966	25
1-Methylpyrene	0.147-.009	Norway - air from N. England	1977	26

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, μg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Benzo[a]pyrene	0.01-75	Urban and non-urban areas	1962	4
	0.73	Near industry handling coal, tar, pitch	1962	4
	2.3-31	Winter, 7 cities, monthly composite	1962	5
	0.3-6.4	Summer, 7 cities, monthly composite	1962	5
	6	"Average" urban air	1967	23
	2.053-0.059	Norway - air from N. England	1977	26
	6-7	Average urban air-Detroit	1965	27
	1	Average suburban air - Detroit	1965	27
	2-37	4 cities in Europe	1967	32
	1.0-11.0	Rome	1975	28
	3.2	College Park, Md.	1976	29
	66	Baltimore Harbor Tunnel	1976	29
	2.68	Budapest	1975	8
	0.31-2.1	Los Angeles	1971	30
	0.5-1.0	London	1976	20
	0.46	Los Angeles	1976	31
	0.11-0.85	Toronto	1975	16
	0.6-8.2	1 site - Sidney, Australia	1965	25
	20-39	London street	1965	25
Benzo[e]pyrene	1-25	12 U.S. cities (est. from data)	1962	24
	5.0	Average U.S. urban atmosphere	1963	24
	12-26	London street	1965	24
	2.635-0.066	Norway - air from N. England	1977	26
	2-25	4 cities in Europe	1967	32

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, μg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Benzo[e]pyrene (Cont'd)	2.6-15.0	Rome	1975	28
	4.6	College Park, Md.	1976	29
	69	Baltimore Harbor Tunnel	1976	29
	1.52	Budapest	1975	8
	0.52-1.2	London	1976	20
	0.90	Los Angeles	1976	31
	0.3-11	20 sites - Ontario (5 samples each location)	1966	25
	4.3-42	20 sites - Ontario (during inversion)	1966	25
	8	Copenhagen (mean annual value)	1966	25
Dibenzo[cd,jk]pyrene (anthanthrene)	1.1	Detroit	1965	24
	Trace - 3	12 U.S. cities (est. from data)	1962	24
	0.26	Average U.S. urban atmosphere	1965	24
	2-6	London street	1965	24
	0.11	Near industry handling coal, tar, pitch	1962	4
	0.423-0.007	Norway - air from N. England	1977	26
	0.1-1.3	Rome	1975	28
	0.23	Los Angeles	1976	31
	1.8 μg/g particulate	Summer - Toronto	1975	16
	2.6 μg/g particulate	Winter - Toronto	1975	16
Dibenzo[a,e]pyrene	100-730 μg/g benzene soluble fraction	Birmingham, Ala. composite	1968	34

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, μg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Indeno[1,2,3-cd]pyrene	1.5-8.2	3 sites in Detroit (6 samples)	1965	24
	1.9-0.062	Norway - air from N. England	1977	26
	3.8-12.8	Rome	1975	28
	1.34	Los Angeles	1976	31
	0.03-1.2	4 sites - Los Angeles	1973	9
Chrysene	1.3-11.6	3 sites in Detroit (6 samples)	1965	24
	Present	6 U.S. cities	1965	24
	3.4-15.0	Rome	1975	28
	4.8	College Park, Md.	1976	29
	106	Baltimore Harbor Tunnel	1976	29
	7.15	Budapest	1975	8
	0.60	Los Angeles	1976	31
	11.6 μg/g particulate	Summer - Toronto	1975	16
	17.5 μg/g particulate	Winter - Toronto	1975	16
	0.2-6.65	1 site - Sidney, Australia (12 samples)	1965	25
	0.4-39.0	14 samples - Rome, Italy	1972	25
	1.3-4.1	5 samples - Rome, Italy	1966	25
Perylene	Trace - 5.0	12 U.S. cities (est. from data)	1962	24
	0.7	Average U.S. urban atmosphere	1965	24
	0.15	Near industry handling coal, tar, pitch	1962	4
	0.191 - trace	Norway - air from N. England	1977	26

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, μg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Perylene (Cont'd)	0.2-1.9	Rome	1975	28
	1.1	Budapest	1975	8
	0.10	Los Angeles	1976	31
	0.034-0.306	Toronto	1975	16
	0.3-0.6	4 samples - Rome	1966	25
	0.01-1.2	4 sites - Los Angeles	1973	9
Benzo[ghi]perylene	2.3-11.5	3 sites in Detroit (6 samples)	1965	24
	2-35	12 U.S. cities (est. from data)	1962	24
	8	Average U.S. urban atmosphere	1965	24
	12-46	London street	1965	24
	0.55	Near industry handling coal, tar, pitch	1962	4
	20 μg/g particulate	Summer - Toronto	1975	16
	30 μg/g particulate	Winter - Toronto	1975	16
	1.971-0.064	Norway - air from N. England	1977	26
	3-26	4 cities in Europe	1967	32
	3.5-21.1	Rome	1975	28
	3.9	College Park, Md.	1976	29
	85	Baltimore Harbor Tunnel	1976	29
	0.86	Budapest	1975	8
	0.7-3.2	London	1976	20
	3.27	Los Angeles	1976	31
	1.0-31	2 sites - London (1954-1964)	1967	25
	1000	1 site - London (inversion - 1957)	1967	25

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, µg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Benzo[ghi]perylene (Cont'd)	6.3-46	20 sites - Ontario (inversion - 1962)	1966	25
	45	Liverpool, England mean value	1966	25
	0.9-9.7	1 site - Sidney, Australia	1965	25
	1.4-3.6	4 samples - Rome	1966	25
	0.2-9.2	4 sites - Los Angeles	1973	9
Coronene	2.3-12.2	2 sites in Detroit	1965	24
	Trace - 8	12 U.S. cities (est. from data)	1962	24
	2	Average U.S. urban atmosphere	1965	24
	4-20	London street	1965	24
	0.05	Near industry handling coal, tar, pitch	1962	4
	0.212-0	Norway - air from N. England	1977	26
	1-6	4 cities in Europe	1967	32
	4.4-18.3	Rome	1975	28
	1.05	Budapest	1975	8
	0.3-1.9	London	1976	20
	2.13	Los Angeles	1976	31
	12.3	Winter - Toronto	1975	16
	8.2	Summer - Toronto	1975	16
	3-48	20 sites - Ontario	1966	25
	0.2-6.4	4 sites - Los Angeles	1973	9
	0.7-1.0	4 samples - Rome	1966	25
Acridine	0.040-0.041	New York City air	1977	22
Methylacridines	ND - 0.007	New York City air	1977	22

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, μg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Benz[a]acridine	0.2	Urban air	1977	24
	3-80 (μg/g benzene soluble fraction)	6 cities urban air	--	5
Benz[c]acridine	1	"Average" urban air	1967	23
	0.6	Urban air	1965	24
	1-30 μg/g BSF	Urban air 6 cities	--	5
	0.1-1.5	50 samples from different U.S. cities	1968	36
Dibenz[a,j]acridine	0.04	Urban air	1965	24
	0-8 μg/g BSF	Urban air	--	5
Dibenz[a,h]acridine	0.1	"Average" urban air	1967	23
	0.08	Urban air	1965	24
Carbazole	1.9	Near industry handling coal, tar, pitch	1962	4
	1.1	Rome	1973	21
Quinoline	0.022-0.069	New York City air	1977	22
	0.6	Rome	1973	21
Methylquinoline	0.033-0.035	New York City air	1977	22
2,6-Dimethylquinoline	0.3	Rome	1973	21
Dimethylquinolines	0.044-0.088	New York City air	1977	22
Ethylquinolines	0.014-0.022	New York City air	1977	22
3C-quinolines	ND-0.010	New York City air	1977	22

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, µg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
Benzo[f]quinoline	0.2	Urban air	1965	24
	0-200 (µg/g benzene soluble fraction)	Urban air (6 cities)	--	5
	0.010-0.011	New York City air	1977	22
Benzo[h]quinoline	0.3	Urban air	1965	24
	1-30 (µg/g BSF)	Urban air (6 cities)	--	5
	0.010-0.013	New York City air	1977	22
11-Indeno[1,2b]quinoline	0.1	Urban air	1965	24
	4-30 (µg/g BSF)	Urban air (6 cities)	--	5
	Trace	New York City air	1977	22
Phenanthridine	0.018-0.022	New York City air	1977	22
Isoquinoline	0.140-0.180	New York City air	1977	22
5 or 8 Methylisoquinoline	0.170-0.310	New York City air	1977	22
Other Methylisoquinolines	0.070-0.076	New York City air	1977	22
Dimethylisoquinolines	ND-0.062	New York City air	1977	22
Ethylisoquinolines	0.068-0.160	New York City air	1977	22
3C-isoquinolines	ND-0.028	New York City air	1977	22
Benzo[f]isoquinoline	0.034-0.110	New York City air	1977	22
4-Azafluorene	0.005	New York City air	1977	22
4-Azapyrene (other isomers)	0.021	New York City air	1977	22
	13.1	Rome	1973	21

Table 5-9 (Cont'd). POLYCYCLIC ORGANIC MATTER IN AMBIENT AIR

Name	Concentration, μg/1000 m^3 (unless otherwise indicated)	Location	Publication Date	Reference
1-Azafluoranthane	0.005 (other isomers)	New York City air	1977	22
	3.0	Rome	1973	21
Benzo[c]cinnoline	1.0	Rome	1973	21
2-Methylindole	2.0	Rome	1973	21
Phenalen-1-one	2	"Average" urban air	1967	23
	0.3-17	Urban site	1969	35
7-H-benz[d,e]anthracen-7-one (benzanthrone)	0-1.01	Non-urban air	1969	13
	0.57-15.52	Urban air	1969	13
	30.5 μg/g particulate	Summer - Toronto	1975	16
	39.1 μg/g particulate	Winter - Toronto	1975	16
	2-48	Urban air	1969	35
	0.9-13	50 samples from different U.S. cities	1968	36
Perinaphthanone	26.5 μg/g particulate	Summer - Toronto	1975	16
	15.9 μg/g particulate	Winter - Toronto	1975	16

5.2 SUMMARY AND CONCLUSIONS

Numerous POM compounds have been detected in ambient air (Table 5-9) with the most extensive data available on benzo[a]pyrene (BaP). The concentrations of BaP are different in various cities and at different times of the year. In general, the concentrations are usually highest during the winter months and lowest during the summer (Figure 5-2) which has been attributed to heating sources of BaP during the winter. The most comprehensive monitoring data on BaP are available from the National Air Surveillance Network (NASN). BaP data are available on the following stations during the indicated years: 1966 to 1970, 250 stations; 1971 to 1976, 40 stations. Trend analysis of the NASN data indicates that BaP concentrations have declined considerably from an annual median value for urban sites of 3.2 ng/m^3 in 1966 to 0.5 ng/m^3 in 1975, an 84 percent decrease. The decline is believed to be due primarily to decreases in coal consumption in residential dwellings and buildings as well as to improved disposal of solid wastes and restrictions on open burning. Concentrations of BaP in urban cities where coke ovens are located were considerably higher (40 to 70 percent) than the non-coke sites. However, this may be due to the fact that most of the coke ovens are located in the north (BaP from heating) and in highly industrialized areas. Recent trends on other POM compounds are not available.

The accuracy of the absolute values of BaP from the NASN data is unknown. The collection efficiencies of glass-fiber filters with high-volume air samples has not been determined and the importance of BaP associated with particles less than 0.3 μm (which could pass through the glass-fiber filter) is unknown. Oxidative loss of BaP during collection is another unknown; BaP degradation may occur between the time the sample is collected and then extracted. In addition, there is experimental evidence indicating that recovery efficiencies are considerably different at summer versus winter temperatures.

Trends for other POM have not necessarily followed the decrease for BaP. For example, in London the concentration of coronene has remained the same while BaP was decreasing. This was attributed to the greater proportion of coronene in auto emissions which are increasing in London.

Whether BaP can be used as an indicator of other POM compounds is not fully established. Some investigators have found good correlations between BaP and other PAH while others have reported little or no relationship. Correlations between BaP and other POM probably vary in different areas and are dependent upon the local POM emission sources which will provide different ratios of individual POM. Because the ratio of many of the individual POM changes in various locations, the use of BaP as an indicator of carcinogenic potential should be reevaluated. A reasonable alternative would appear to be some form of multi-component analysis that would include some of the other carcinogenic or tumor-promoting POM.

A little information on the nitrogen heterocycles, aza- and imino-arenes, is available. In general, these compounds are found at concentrations 10 to 100 times lower than the PAH, although a recent study in Rome found 1 to 10 ng/m^3 of individual aza-arene compounds. The monitoring trends of aza-arene concentrations are unknown. Whether they are increasing or decreasing in concentration and whether there is a seasonal fluctuation is also undetermined. They have been detected near coal conversion plants, but their major emission source is unknown.

A recent study in Norway has demonstrated that at least 20 PAH associated with particulate matter are stable enough in the atmosphere to travel long distances. Four of these compounds, including BaP, have been shown to have carcinogenic activity.

In summary, the concentrations of at least 25 PAH and 32 aza-arenes or oxygen-substituted POM have been determined in ambient air which suggests that they are stable enough to come in contact with humans, animals, and plants. The concentration of BaP has decreased significantly over the last 10 years (84 percent decrease), but data in the United States on whether other POM compounds have followed this trend are unavailable. Data in other countries have indicated that POM compounds (e.g., coronene), which are found in relatively large proportions in automotive emissions, are remaining constant while BaP levels are decreasing.

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6. HEALTH EFFECTS

6.1 PHARMACOKINETICS

There are no data available concerning the pharmacokinetics of POM in humans. Nevertheless, it is possible to make limited assumptions based on the results of animal studies conducted with several POM, particularly BaP. The different patterns of metabolism for POM in human and animal tissues has been especially well-studied, and has contributed significantly to an understanding of the mechanisms of POM-induced cancer.

6.1.1 Absorption

The demonstrated toxicity of POM by oral and dermal administration indicates that they are capable of passage across epithelial membranes.[1] The high lipid solubility of compounds in this class supports this observation. Early studies conducted on the intestinal absorption of BaP confirmed that transfer across the gut readily occurs and, moreover, that tissue accumulation is exponential as BaP concentrations increase.[2] These results are consistent with a mechanism involving physical adsorption of BaP to the intestinal mucosa as a first step. A unilayer is presumably formed at saturation concentrations and a multilayer at higher BaP levels. Increases in the medium concentration of BaP above the saturation level would thereby permit an exponential transport into the cells by passive diffusion. The authors indicated further that the intestinal transport of MCA, DMBA, chrysene, and benz[a]anthracene was similar to BaP. Assuming that the ability of various organs to absorb BaP (and other POM) also follows an exponential relationship, relatively small differences in the magnitude of environmental exposures could be expected to exert a dramatic influence on the body burden of POM. Such a relationship has been demonstrated for the accumulation of BaP in adipose tissue.[2]

The absorption of POM through the lungs has particular relevance to environmental exposure situations. However, the major health-related effects of inhalation exposure involve local lesions in the respiratory tract; unfortunately, few investigators bother to measure systemic levels of POM. Nevertheless, it is known that BaP administered intratracheally to rats appears in the tissues with the same pattern of distribution as when given parenterally.[3] Vainio and coworkers[4] recently showed that unchanged BaP quickly appears in the perfusion fluid of isolated perfused rat lungs following intratracheal administration of a 200 nmole dose. Pretreatment of rats with an intraperitoneal injection of MCA to induce microsomal enzyme activity caused an increase in the amount of water-soluble metabolites of BaP appearing in the perfusate. An increased covalent binding of BaP metabolites to the lung tissue also accompanied the MCA-mediated induction of microsomal monooxygenases. The significance of tissue-binding of POM metabolites is discussed in Section 6.2.6 of this report.

Because POM generally reaches the lung under environmental conditions by adsorption on carrier particles, the extent of particle deposition in the lungs and the elution of the chemical from the particle have an important bearing on its biological effect. In this regard, Creasia and coworkers[5] showed that when BaP is adsorbed to large carbon particles (15-30 μm) and instilled into the lungs, 50 percent of both the BaP and the carrier particles were cleared from the lungs in four to five days. Little carcinogen was released from the carbon particles in this case, and therefore contact with the respiratory epithelium (and carcinogenicity) was low. With smaller carbon particles (0.5-1.0 μm), however, 50 percent particle clearance was not achieved until seven days after administration. In this case, 15 percent of the adsorbed BaP was eluted from the particles and left free to react with the respiratory tissues. In the complete absence of carrier particles, BaP was cleared from the lung at 20 times the rate of adsorbed BaP. This observation may explain the difficulty in producing experimental pulmonary tumors with BaP without the use of carrier particles. Other investigators[6] confirmed that carbon particle size affects BaP retention in the lung, but also demonstrated that BaP retention was not affected by particle size when adsorbed on ferric oxide or aluminum oxide.

The elution of BaP from carbon particles instilled in the respiratory tract of mice can be enhanced if the particles are instilled during the acute phase of respiratory infection.[7] The fate of the eluted BaP in the lung was not determined although the author predicted that rapid elimination would occur, thus reducing the risk of pulmonary carcinogenesis.

6.1.2 Distribution

Regardless of its route of administration, POM, once absorbed, becomes localized in a wide variety of body tissues. The distribution of radioactivity derived from ^{14}C-BaP in the rat and mouse was determined following subcutaneous, intravenous, and intratracheal administration.[3] The pattern of distribution was found to be similar in all cases, except for high local pulmonary concentrations following intratracheal administration (Tables 6-1 and 6-2). Concentrations of BaP-derived radioactivity in the liver reached a maximum within only 10 minutes after injection and represented 12 percent of the total dose. Radioactivity in the liver was reduced to one to three percent of the administered dose within 24 hours. Similarly, maximum blood levels of BaP following intravenous injection were reached very quickly, and radioactivity became barely detectable after 10 minutes. Minimal tissue localization of BaP and/or its metabolites occurred in the spleen, kidney, lung, and stomach; maximum radioactivity derived from labeled BaP was recovered in the bile and feces. Levels of radioactivity in fat, skin, and muscle were not determined, nor was the amount of unchanged BaP measured in any tissue. Bock and Dao[8] later showed that relative to other tissues, unmetabolized BaP was extensively localized in the mammary gland and general body fat after a single feeding of the carcinogen (10-30 mg). This accumulation of BaP was greater than that resulting from administration of 3-methylcholanthrene (MCA), 7,12-dimethylbenz[a]anthracene (DMBA), or phenanthrene (Table 6-3). In all cases, the level of carcinogen achieved in the tissue was directly related to the dose administered and was dependent upon the use of a lipid vehicle. The authors suggested that the mammary fat pad may act as a carcinogen trap, which slowly releases the unchanged hydrocarbon to the glandular cells. In the rat, the glandular tissue is a common site for POM-induced breast cancer.

A more complete study on the tissue distribution of MCA was reported in 1959 by Dao and coworkers[9] after it was shown that oral administration of MCA

Table 6-1. DISTRIBUTION AND RATE OF ELIMINATION OF RADIOACTIVITY AFTER INTRAVENOUS INJECTION OF ^{14}C-BENZO[a]PYRENE INTO RATS[a,3]

Site	Time after injection of aqueous suspension, hr.		Time after injection of human plasma solution, hr.	
	4	24	4	10
	% of injected dose			
Feces	0.35	8.2	2.8	37.4
Stomach	0.69	3.7	0.5	0.31
Intestine	23.5	43.8	47.0	25.5
Liver	3.9	1.3	4.2	2.5
Urine	4.3	12.9	4.5	8.3
Kidney	1.05	0.9	1.9	0.99
Lung	0.5	0.27	0.3	0.69
Spleen	1.0	0.06	0.68	0.15
Other organs (testes and myocardium)	0.8	0.07	0.74	0.16
Percent recovered	36.09	71.20	62.62	76.00

[a] Dose: 30 µg ^{14}C-benzo[a]pyrene in 0.9 ml vehicle.

Table 6-2. DISTRIBUTION AND RATE OF ELIMINATION OF RADIOACTIVITY AFTER INTRATRACHEAL INSTILLATION OF ^{14}C-BENZO[a]PYRENE INTO RATS[a] [3]

Site	Time after instillation of aqueous suspension, hr.		Time after instillation of triethylene glycol solution, hr.
	1	24	4
	% of administered dose		
Feces	0	28.0	17.2
Stomach	0	0	0
Intestine	37.0	11.1	44.2
Liver	1.3	4.3	5.8
Urine	0	1.2	8.8
Kidney	0	2.4	2.6
Lung	43.2	38.6	17.7
Spleen	0	0	0
Other organs (testes and myocardium)	0.08	0.43	0.29
Percent recovered	81.58	86.03	96.59

[a] Dose: 25 μg ^{14}C-benzo[a]pyrene in 0.3 ml vehicle.

Table 6-3. EFFECT OF MOLECULAR STRUCTURE ON HYDROCARBON LOCALIZATION IN MAMMARY GLAND AND FAT[a] [8]

Hydrocarbon	Number of rats	Hydrocarbon concentration, μg/gm ± S.E.[b]	
		Mammary fat	Fat
3-Methylcholanthrene	30	18.0 ± 1.6	39.2 ± 3.9
Benzo[a]pyrene	10	29.1 ± 4.0	55.9 ± 5.3
7,12-Dimethylbenz[a]anthracene	10	23.4 ± 1.7	39.3 ± 4.4
Phenanthrene	5	1.8 ± 0.1	2.8 ± 0.3

[a] Levels of hydrocarbon were determined 24 hours after a single intragastric feeding of 30 mg test compound in 1 ml of sesame oil. Quantitative assays were conducted by fluorescence measurements of the crude benzene extract of tissue containing hydrocarbon.

[b] ± Standard deviations.

produces mammary cancers. A quantitative analysis of MCA in rat tissues 24 hours after its oral administration demonstrated that the hydrocarbon was mainly localized in the fatty and breast tissues (Table 6-4). In virgin animals, levels of MCA were higher in fat than in breast tissues, whereas in lactating rats this relationship was reversed. Moreover, significant amounts of MCA appeared in the milk at 24 hours after administration (Table 6-5). On the other hand, West and Horton[10] reported that the transfer of ingested BaP and MCA to the milk of rabbits and sheep was far less than that in rats; a phenomenon which is probably common to all ruminants.

Detailed studies concerning the tissue distribution of tritiated DMBA produced similar results as for MCA.[11] Twenty-four hours after receiving a single oral dose of 30 mg ^{3}H-DMBA at two levels of radioactivity (26 μCi and 2600 μCi), the greatest amount of activity recovered from rat tissues was in the perirenal fat. Moderate levels of radioactivity were also detected in kidney, liver, and mammary tissue. Radioactivity in the fat and mammary gland persisted for at least 3 days and represented predominantly unmetabolized DMBA. In contrast, most of the radioactivity localized in the liver was due to the presence of polar DMBA metabolites, some of which were bound to nucleic acids and cellular proteins.

The tissue distribution of ^{3}H-dibenzo[g,c]carbazole (DBcgC) following intratracheal instillation was qualitatively and quantitatively similar to BaP.[12] Except for high local concentrations of DBcgC in the lungs, the greatest amount of radioactivity was localized in the intestines of treated hamsters (Table 6-6).

Table 6-4. LEVELS OF ORALLY ADMINISTERED 3-METHYLCHOLANTHRENE IN TISSUES OF 50-DAY-OLD VIRGIN FEMALE RATS[9]

Tissue	Level of 3-methylcholanthrene, μg/g tissue[a,b]
Breast, rat 1	15
Fat, rat 1	70
Breast, rat 2	12
Fat, rat 2	17
Breast, rat 3	24
Fat, rat 3	38
Brain, pooled	3.5
Lung, pooled	2.1
Kidney, pooled	1.1
Thymus, pooled	1.4
Liver, pooled	0.3
Muscle, pooled	0.04
Heart, pooled	NF[c]
Spleen, pooled	NF
Uterus, pooled	NF
Ovaries, pooled	NF
Adrenals, pooled	NF
Pituitaries, pooled	NF

[a] MCA was given as a single 30 mg dose in 1 ml of sesame oil. Animals were sacrificed 24 hours later and levels of MCA in crude benzene extracts of tissue determined by fluorescence.

[b] Total content of 3-methylcholanthrene was less than 0.1 μg in pooled tissues of 3 rats.

[c] Not found.

Table 6-5. LEVELS OF ORALLY ADMINISTERED 3-METHYLCHOLANTHRENE IN BREAST AND FAT OF VIRGIN RATS AND IN MILK, BREAST, AND FAT OF LACTATING RATS[a] [9]

	Rat No.	Tissues	Weight, g	3-Methylcholanthrene, μg/g tissue[b]
Virgin rats 50 days old	1	Breast	2.6	6.5
		Fat	0.9	7.1
	2	Breast	2.1	6.4
		Fat	0.9	10.9
Lactating rat (7 days postpartum)	1	Breast	11.5	17
		Fat	1.7	11
		Milk (fetus 1)		23
		(fetus 2)		13

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[a] One single feeding of 3-methylcholanthrene of 10 mg in 1 ml of sesame oil was given and animals were sacrificed 24 hours later.

[b] Crude benzene extracts of tissue were analyzed in a spectrophotofluorometer using either 295 mμ or 355 mμ excitation light.

Table 6-6. DISTRIBUTION AND ELIMINATION OF RADIOACTIVITY AFTER INSTILLATION OF ^{3}H-DIBENZO[c,g]CARBAZOLE IN SYRIAN HAMSTERS[a] [12]

Site	Percent instilled dose recovered at:		
	1 hr.	3 hr.	6 hr.
Trachea	11 ± 1.2	4.0 ± 0.35	3.0 ± 0.34
Lungs	63 ± 2.2	32 ± 4.1	18 ± 2.6
Liver	1.4 ± 0	4.6 ± 0.40	4.3 ± 0.15
Kidneys	0.0	2.0 ± 0.36	1.8 ± 0.11
Brain	0.0	0.7 ± 0.06	0.7 ± 0.01
Intestines (large and small)	11 ± 1.4	31 ± 2.6	35 ± 5.8
Fat	0.0	0.6 ± 0.15	0.6 ± 0.04
Urine	0.0	0.6 ± 0.07	3.2 ± 0.13
Feces	0.0	8.2 ± 0.12	15.6 ± 1.45
Total % recovered	86.4 ± 5.44	83.7 ± 4.65	82.2 ± 4.23

[a] Animals were treated once with 3 mg ^{3}H-dibenzo[c,g]carbazole suspended with Fe_2O_3 particles in 0.2 ml saline.

Transplacental passage of POM is a critical factor in determining the risk for transplacental toxicity and carcinogenesis resulting from maternal exposures. The ability of BaP, DMBA, and MCA to cross the placenta in rats was investigated following a single intragastric dose of 200 mg/kg of the hydrocarbon as a suspension in sunflower oil.[13] With DMBA, the concentration of the carcinogen in fetal tissues reached a maximum (1.53 to 1.6 μg/g) 2 to 3 hours after maternal administration. At 2 hours, the ratio of DMBA concentrations in maternal liver, placenta, and fetuses was 10:1.5:1. Passage of BaP into the fetus was even greater than for DMBA. MCA, on the other hand, crossed the placenta in only trace amounts. These data are summarized and compared in Table 6-7. Intravenous injection of a 25 mg/kg dose of DMBA produced similar fetal tissue levels as the intragastric administration of 200 mg/kg DMBA.

Limited studies have been conducted with human cells to characterize the pattern of cellular uptake and distribution of POM. Ekelman and Milo[14] exposed cultured human neonatal foreskin fibroblasts to radiolabelled BaP and DMBA. Radioactivity was first concentrated at the cell membrane, then randomly distributed throughout the cytoplasm, and ultimately (after 12 hours) localized at the nuclear membrane or in the nucleus. Their results further indicated that BaP was first bound to a cytoplasmic receptor protein and subsequently transferred to the nucleus. On the other hand, DMBA was absent from a charcoal-treated cytoplasmic fraction, indicating that protein binding did not occur, and consequently DMBA was not extensively localized in the nucleus. The authors postulated that differences in binding of POM to cytoplasmic protein could account for the apparent resistance of human cells to

Table 6-7. CONCENTRATION (μg/g) OF DMBA, BaP, AND MCA IN TISSUES OF 21-DAY FETUSES, THEIR PLACENTAS, AND THE LIVER OF PREGNANT RATS 3 HOURS AFTER ADMINISTRATION OF THE COMPOUNDS IN A DOSE OF 200 mg/kg BY GASTRIC TUBE[13]

Carcinogen administered	Fetuses		Placentas		Liver	
	Mean and limits of variations	Number of analyses	Mean and limits of variations	Number of analyses	Mean and limits of variations	Number of analyses
DMBA	1.53 (0.63-3.50)	4	1.28 (0.86-1.60)	4	8.80 (5.50-11.80)	4
BaP	2.77 (1.67-5.00)	5	3.94 (2.24-6.15)	5	23.50 (11.10-50.20)	4
MCA	0.0013 (traces-0.002)	6	0.009 (traces-0.03)	6	0.10 (0.03-0.22)	6

many carcinogens. In support of this view, the authors cited unpublished evidence that human diploid foreskin cells were successfully transformed by BaP but not by DMBA.

Theoretical studies on the uptake of POM by model membrane phospholipid vesicles suggest a role for particle-mediated membrane uptake of POM as a factor in carcinogenesis.[15,16] Chrysene and BaP were shown to enter phospholipid bilayers much more rapidly when adsorbed on silica or asbestos. When adsorbed to asbestos, BaP was transported into membranes more rapidly than when adsorbed to silica, despite the fact that the asbestos sample had 60-fold less surface area than the silica sample. The significance of these findings was related to the possible involvement of environmental particulates in aiding the transport of POM into cell membranes and increasing the effective concentration of carcinogenic material available for microsomal activation in the cytoplasm. Evidence was cited regarding the apparent interaction of POM in cigarette smoke with asbestos inhalation in the production of lung cancer as support for the role of particulates in chemical carcinogenesis.

6.1.3 Elimination

As long ago as 1936, it was recognized that various polycyclic aromatic hydrocarbons were primarily excreted through the hepatobiliary system and the feces.[17,18] With the advent of radiotracer methodologies, it became possible to study more quantitatively the elimination of POM.[3,19] Patterns of ^{14}C-BaP excretion were determined in both intact rats and in rats with biliary fistulas.[3] When BaP was given intratracheally, or by subcutaneous and intravenous injection, maximum excretion of radioactivity always occurred through the feces (Tables 6-1 and 6-2). In addition, the bile contained significant amounts of radioactivity, which was enhanced by increasing the administered dose of BaP until a saturation level was reached at 150 μg BaP (intravenous dose). Radioactivity was detectable in the bile for 24 hours following treatment, and a total of 1 percent of the administered BaP was recovered unchanged. The total recovery of radioactivity in BaP-treated rats having biliary fistulas is shown in Table 6-8. The importance of enterohepatic circulation in the excretion of BaP was indicated by the fact that urinary excretion of radioactivity was reduced from 7 to 14 percent of the total dose in intact rats to 3 to 4 percent of the total dose in rats with biliary fistulas.

The influence of route of administration on the rate of POM elimination is likely to be an important determinant in quantitative studies. Following the intragastric administration of MCA to rats, 82 percent of the dose is excreted in the feces within 24 hours.[20] However, when injected intraperitoneally, only 30 percent of the dose appeared in the feces after 3 days. Assuming that MCA is readily absorbed through the gastrointestinal tract (a valid assumption based on the data of Rees and coworkers[2]), it was apparent that rapid absorption and metabolism accounted for the lack of appreciable MCA retention. It may thus be postulated that liver "first-pass" metabolism as well as biotransformation by enzymes of the intestinal mucosa represent an effective means of handling ingested POM's. On the other hand, POM's reaching the circulation by absorption through the lungs or skin will not make an initial passage through the liver and thus are more likely to reach internal target organs in the parent form. Since environmental exposures are very

Table 6-8. DISTRIBUTION OF RADIOACTIVITY IN RATS WITH BILIARY FISTULAS AFTER INTRAVENOUS INJECTION of ^{14}C-BENZO[a]PYRENE IN PLASMA SOLUTION[3]

Dose, µg	Bile flow rate, µl/minute	Duration of experiment, hours	Recovery in bile, % of injected dose			
			1 hr.	2 hr.	3 hr.	Total
19	7.2	4	17	9	9	39
26	18.9	12	21	19	9	78
45	18.0	12	27	18	7	75
134	12.6	14	26	30	13	96
400	15.3	24	4	3	3	32

important by inhalation, it cannot be assumed that excretion of POM will be sufficiently rapid to prevent their accumulation in various tissues.

When administered by subcutaneous injection or by application to the skin of female mice, BaP, MCA, and DBahA are eliminated almost entirely by the feces.[19] However, the rate of disappearance from the site of application or injection varies considerably in the decreasing order BaP>MCA>DBahA. In particular, the elimination of DBahA when applied to the skin is extremely slow, and none of the compound or its metabolites are found in the feces. Poor dermal absorption of DBahA probably accounts for this observation since binding of metabolites to skin proteins was not extensive, nor is DBahA known as a potent skin carcinogen.

Disappearance of BaP from the blood and liver of rats following a single intravenous injection was very rapid.[21] The concentration of BaP in the blood one minute after a 10 μg injection was 193 ± 29 ng; after five minutes the concentration of BaP in the blood was 31 ± 1 ng. Similarly, in the liver the half-time for BaP disappearance was about 10 minutes. In both blood and liver, however, the initial rapid elimination phase was followed by a slower disappearance phase, lasting six hours or more. In the same experiment, disappearance of BaP from the brain was slower than from blood or liver, and the concentration of BaP in fat increased during the six hour observation period. Schlede and coworkers[21] concluded that a rapid equilibrium occurs for BaP between blood and liver, and that rapid disappearance from the blood is due to both metabolism and distribution into tissues. This contention is supported by data[22] showing that pretreatment with BaP (which induced microsomal enzyme activity) accelerates both the rate of BaP disappearance from all tissues and the excretion of BaP metabolites into the bile. The ability of BaP to stimulate its own metabolism may have important implications for human situations where lifelong exposure to POM is known to occur. Furthermore, in the pregnant rat pretreatment with BaP, MCA, chrysene, benz[a]anthracene, or DBahA had a marked stimulatory effect on the in vitro metabolism of a subsequent dose of BaP in maternal liver, placenta, and fetal liver.[23]

Elimination of BaP from the lung is generally regarded to be influenced by the presence of carrier particles to which the hydrocarbon is adsorbed. Most investigators have found that BaP retention by the lung is increased when administered with particulate material.[6,24] Pelfrene[25] administered single intratracheal instillations (3 mg) of BaP to hamsters either alone or in combination with talc (3 or 9 mg). He observed an increased retention time for BaP in the lungs of hamsters also receiving talc (Table 6-9). The mechanism for talc-induced retention of BaP was not explored, although the possibility of saturation of the macrophagic system by talc was offered as an explanation for reduced clearance.

However, recent studies have also indicated that intratracheal instillation of BaP to hamsters in combination with ferric oxide did not increase carcinogen retention over that obtained with BaP alone.[26] Moreover, it is now known that carrier particles are not an absolute requirement for the induction of respiratory tract tumors by BaP.[27] Rather than affecting retention, it was suggested that carrier particles determine tissue localization and thereby tumor development. A crucial factor in determining adequate lung retention of

Table 6-9. AMOUNTS OF B(a)P DETECTED IN HAMSTER LUNGS FOLLOWING INTRATRACHEAL INSTILLATION[25]

	Amounts found at post-instillation time of:[1] Hours						
Treatment	0	3	6	24	48	96	168
3 mg B(a)P	59.3 ± 3.8%[2]	56.1 ± 3.7%	29.5 ± 1.7%	5.7 ± 0.6%	0		
3 mg B(a)P+ 3 mg talc	69.1 ± 2.3%	59.1 ± 5.5%	60.0 ± 6.0%	25.5 ± 2.9%	20.6 ± 1.3%	10.4 ± 2.4%	0
3 mg B(a)P + 9 mg talc	79.8 ± 8.3%	65.5 ± 12%	62.6 ± 6.6%	38.1 ± 3.2%	24.1 ± 4.1%	5.3 ± 2.0%	0

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[1] Time from instillation at which animals were killed.

[2] Standard deviation. Figures are the average of data from 6 animals per group. They represent the amount of B(a)P detected in the lungs at given time intervals (i.e., the percentage of that administered in a single intratracheal instillation).

carcinogens for tumor development is the concommitant exposure to agents which inhibit ciliary activity.[28] The tracheobronchial epithelium is normally protected by a mucous blanket and ciliated cells. These cells propel foreign materials captured in the mucous flow upward and out of the respiratory tract to be swallowed and excreted. It has been known for many years that irritants (e.g., SO_2, polluted air, cigarette smoke) can inhibit ciliary activity, thus increasing the time available for elution of POM's from soot particles in the lungs. This process can be expected to markedly influence the response to carcinogenic POM.

On the other hand, it should not be assumed that particles cleared from the lungs by ciliary activity are necessarily removed from the body. These particles are usually swallowed and thus present the opportunity for POM absorption via the gastrointestinal tract, a process which is known to result in tumor formation (see Section 6.4).

A thorough examination has been conducted on the clearance of intratracheally instilled ^{3}H-BaP (5 mg total dose) from various tissues of the hamster.[29] The effect of the presence of adsorbents (asbestos, carbon black) was also studied. Elimination of radioactivity from the lung was found to be biphasic; an initial rapid phase lasted about 2 weeks and was not significantly influenced by the presence of adsorbents (Figure 6-1). During this phase, 99 percent of the administered dose was eliminated from the respiratory tract. In the second (slow) phase of elimination, a significant retention of radioactivity became evident only among animals given ^{3}H-BaP together with an adsorbent. This accumulation of ^{3}H-BaP-derived radioactivity in the lung,

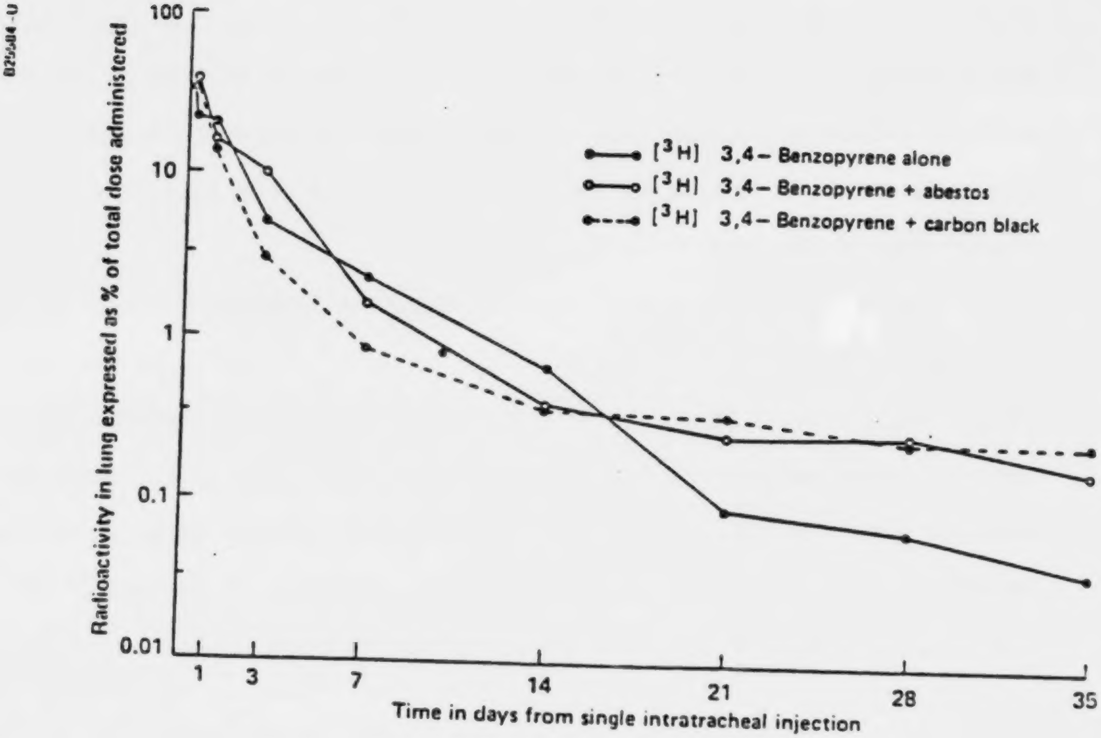


Figure 6-1. Clearance of radioactivity derived from $[^3H]$3,4-benzopyrene in the lung.[29]

although small, was considered toxicologically significant. The levels of radioactivity in other organs and in blood, urine, and feces were not apparently influenced by co-administered adsorbents, but did appear to follow a biphasic pattern of elimination (Figures 6-2 to 6-6). However, blood and fecal levels of radioactivity dropped off more rapidly in those animals given ^{3}H-BaP with carbon black. These results (and those of numerous similar studies) are presented with the assumption that the pattern of distribution and elimination of the tritium label is an accurate reflection of the BaP molecule and/or its metabolites. In most cases, no attempt is made to identify metabolites or determine the extent of tritium loss into body water or of bioexchange of the label.

Excretion of intratracheal doses of DBcgC in hamsters proceeds in much the same way as for BaP.[12] The principal route of excretion is via the feces. Retention of DBcgC by the respiratory tissues was dependent on the administration vehicle (saline or water suspension), but in all cases was less than for BaP. Only 6 percent of an intratracheal dose of DBcgC in saline remained in the lung after 18 hours, whereas with BaP, 25 percent of the administered compound was still present after 18 hours.

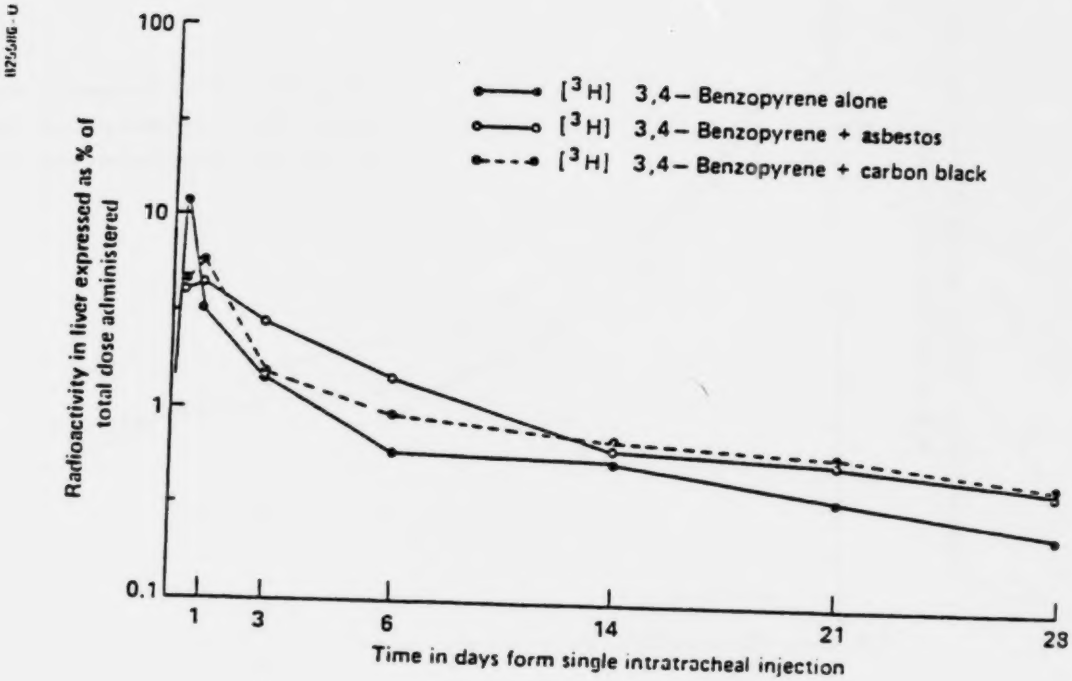


Figure 6-2. Clearance of radioactivity derived from [^{3}H]3,4-benzopyrene in liver.[29]

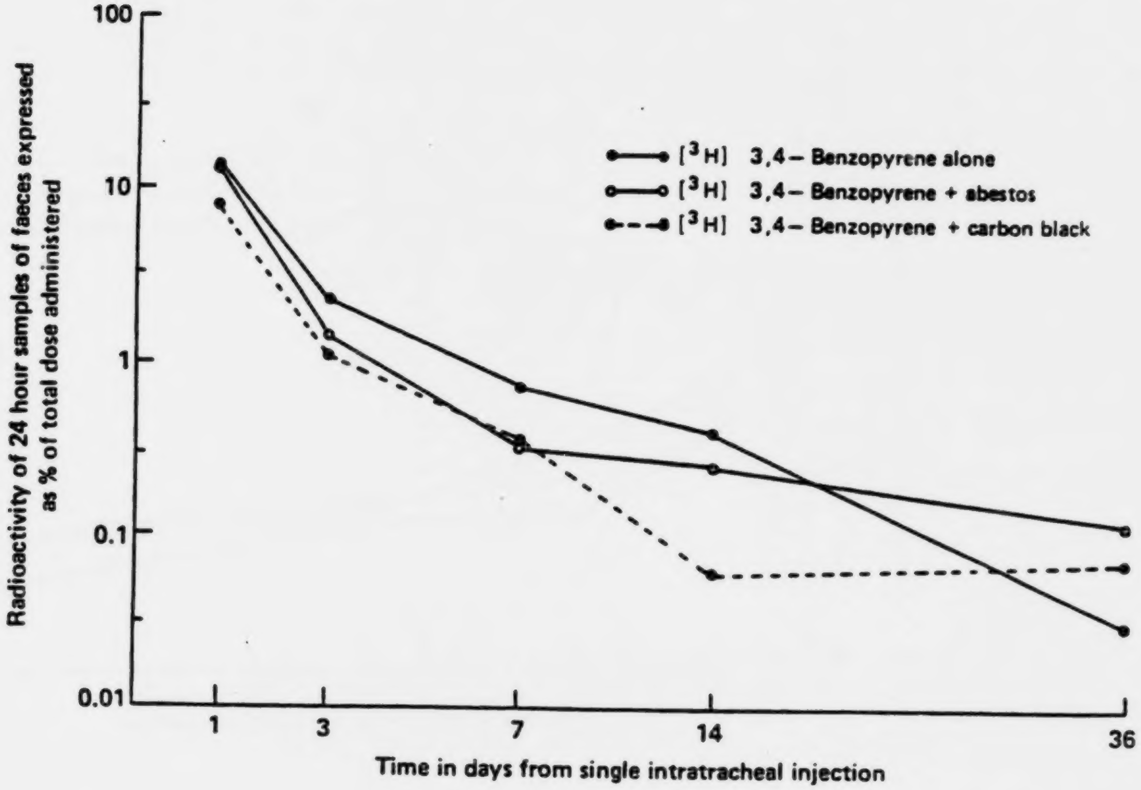


Figure 6-3. Excretion of radioactivity derived from [^{3}H]3,4-benzopyrene in feces.[29]

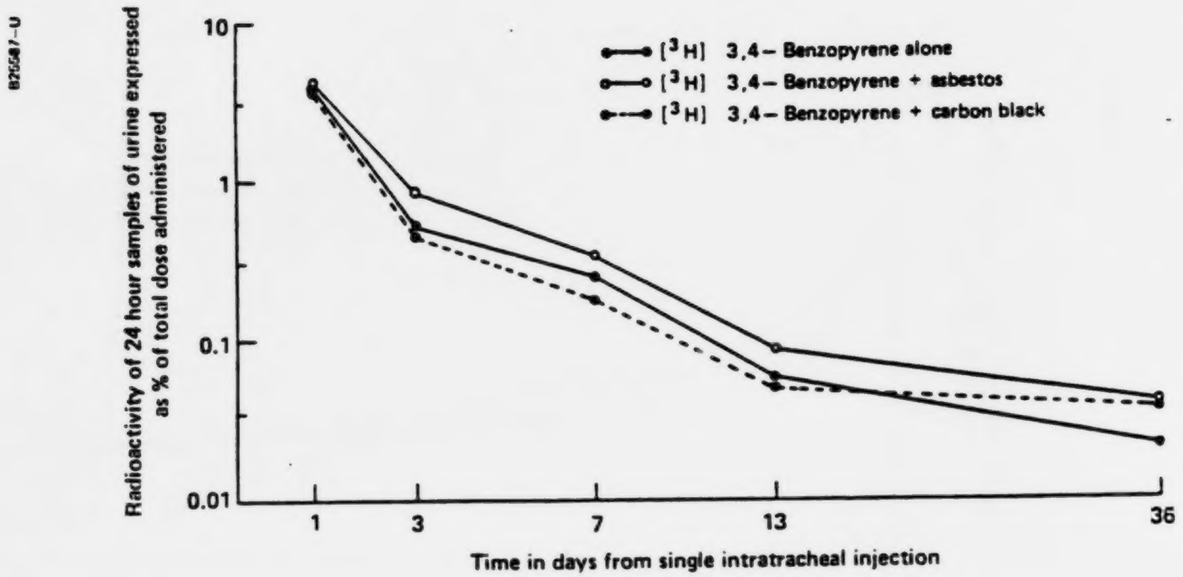


Figure 6-4. Excretion of radioactivity derived from $[^3H]$3,4-benzopyrene in urine.[29]

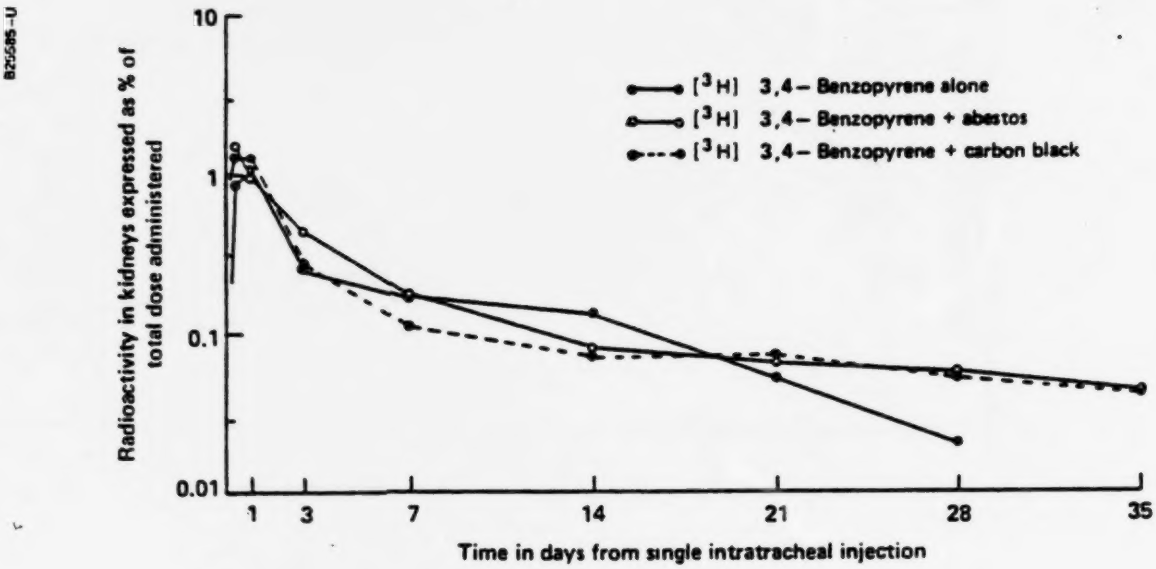


Figure 6-5. Clearance of radioactivity derived from [^{3}H]3,4-benzopyrene in kidneys.[29]

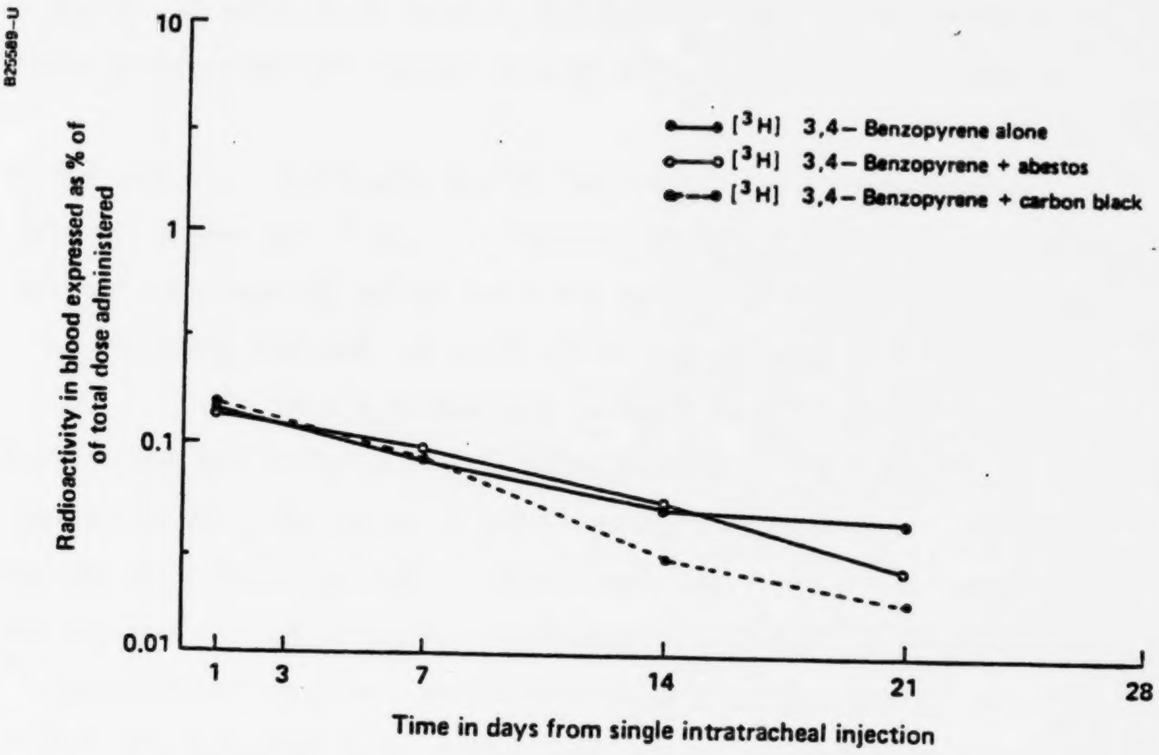


Figure 6-6. Blood level of radioactivity derived from [^{3}H]3,4-benzopyrene.[29]

6.2 METABOLISM

In the past, the relative lack of chemical reactivity for tumorigenic POM's has been puzzling in light of their dramatic biological effects. Early attempts to explain the carcinogenicity of various POM's utilized physicochemical calculations.[30] These early hypotheses were based on the assumption that those regions of the molecule favoring substitution or addition reactions would preferentially react with critical cellular target sites to initiate a carcinogenic transformation. This concept, however, did not prove successful for POM.

More recently it was learned that POM are metabolized via enzyme-mediated oxidative mechanisms to form reactive electrophiles.[31] For many of the POM, certain "bioactivated" metabolites are formed having the capability for covalent interaction with cellular constituents (i.e., RNA, DNA, proteins) and ultimately leading to tumor formation (see Section 6.2.6).

The obligatory involvement of metabolic activation for the expression of POM-induced carcinogenesis has prompted the investigation of POM metabolism in numerous animal models and human tissues. From these studies has emerged an understanding of the general mechanisms involved in POM biotransformation. It is now known that POM are metabolized by the cytochrome P-450-dependent microsomal mixed-function oxidase (MFO) system, often designated aryl hydrocarbon hydroxylase.[32,33,34,35] The activity of this enzyme system is readily inducible by exposure to chemicals and is found in most mammalian tissues, although predominantly in the liver.[36,37,38,39,40,41,42] The MFO system is involved in the metabolism of endogenous substrates (e.g., steroids) and the detoxification of many xenobiotics. Paradoxically, however, the MFO system also catalyzes the formation of reactive epoxide metabolites from certain POM, possibly leading to carcinogenesis in experimental mammals[31,43-48] (see Section 6.4.2). A second microsomal enzyme, epoxide hydrase, converts epoxide metabolites of POM to vicinal glycols, a process which may also play a critical role in carcinogenic bioactivation. Figure 6-7 presents a schematic representation of the various enzymes involved in activation and detoxification pathways for BaP. At present this also appears to be representative of the general mechanism for POM metabolism.

A discussion of the metabolism of POM in mammalian species, including man, is best approached by examining in detail the chemical fate of the most representative and well-studied compound in the POM class, namely BaP. The metabolism of BaP has been extensively studied in rodents, and the results of these investigations provide useful data which can be directly compared to and contrasted with the results of more limited studies employing human cells and tissues. Therefore, separate discussions are presented below based upon the available experimental evidence regarding POM metabolism in general, and BaP metabolism in particular, in both animals and man.

6.2.1 Metabolism of POM in Animals

The metabolites of POM produced by microsomal enzymes in mammals can arbitrarily be divided into two groups on the basis of solubility. In one group are those metabolites which can be extracted from an aqueous incubation mixture by an organic solvent. This group consists of ring-hydroxylated products, such as phenols and dihydrodiols,[49,50] and hydroxymethyl derivatives of those POM having aliphatic side chains, such as 7,12-dimethylbenz[a]-anthracene[51] and 3-methylcholanthrene.[52,53] In addition to the hydroxylated

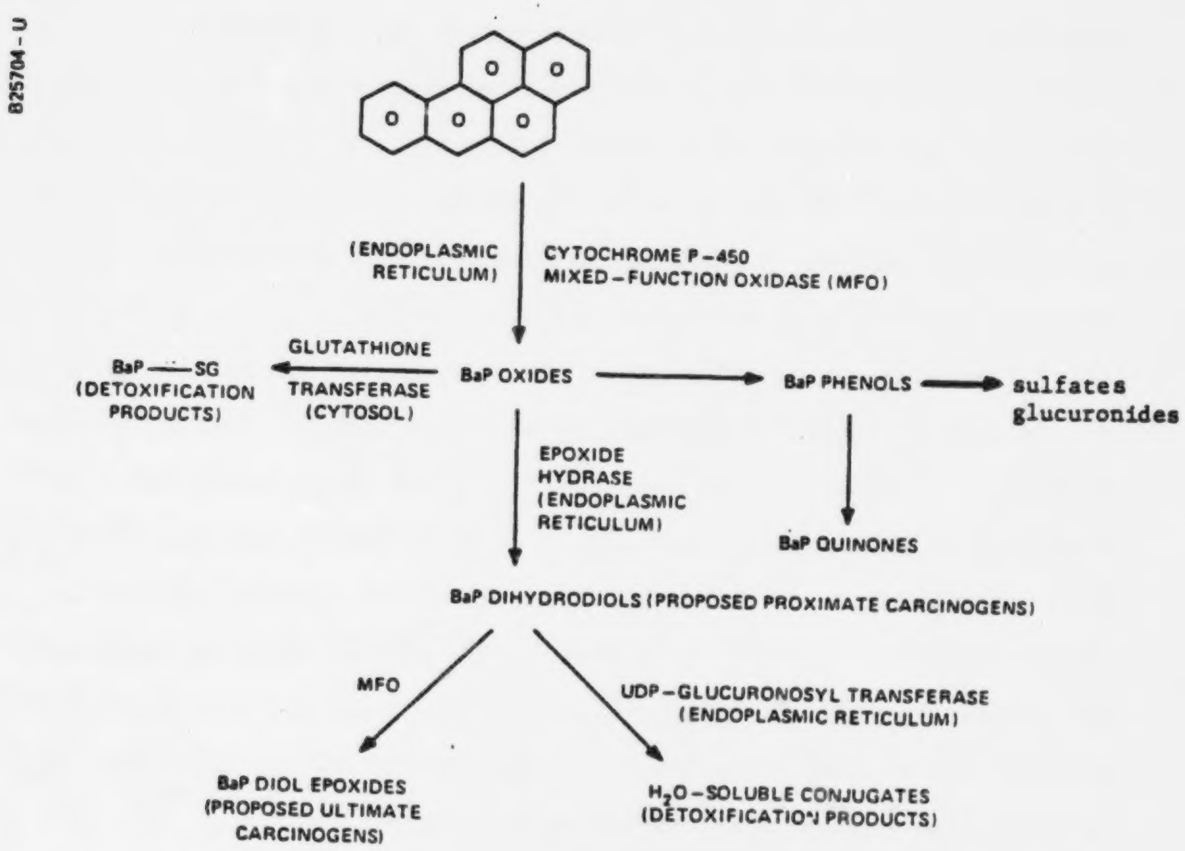

B25704-U
(ENDOPLASMIC RETICULUM)
CYTOCHROME P-450 MIXED-FUNCTION OXIDASE (MFO)
BaP OXIDES
GLUTATHIONE TRANSFERASE (CYTOSOL)
BaP—SG (DETOXIFICATION PRODUCTS)
BaP PHENOLS
sulfates
glucuronides
EPOXIDE HYDRASE (ENDOPLASMIC RETICULUM)
BaP QUINONES
BaP DIHYDRODIOLS (PROPOSED PROXIMATE CARCINOGENS)
MFO
UDP-GLUCURONOSYL TRANSFERASE (ENDOPLASMIC RETICULUM)
BaP DIOL EPOXIDES (PROPOSED ULTIMATE CARCINOGENS)
H2O-SOLUBLE CONJUGATES (DETOXIFICATION PRODUCTS)

Figure 6-7. Enzymatic pathways involved in the activation and detoxification of BaP.

metabolites are quinones, produced both enzymatically by microsomes and nonenzymatically by air oxidation of phenols. Labile metabolic intermediates such as epoxides can also be found in this fraction.[43,44,45,54]

In the second group of POM metabolites are the water-soluble products remaining after extraction with an organic solvent. Many of these derivatives are formed by reaction (conjugation) of hydroxylated POM metabolites with glutathione, glucuronic acid, and sulfate.[55,56] Enzyme systems involved in the formation of water-soluble metabolites include glutathione S-transferase, UDP-glucuronosyl transferase, and sulfotransferases.[43,57,58] Conjugation reactions are believed to represent detoxification mechanisms, although this group of derivatives has not been rigorously studied.

The metabolite profile of BaP which has recently been expanded and clarified by the use of high pressure liquid chromatography is depicted in Figure 6-8. This composite diagram shows three groups of positional isomers, three dihydrodiols, three quinones, and several phenols. The major BaP metabolites found in microsomal incubations are 3-hydroxy-BaP, 1-hydroxy-BaP, 7-hydroxy-BaP, and 9-hydroxy-BaP. The BaP-4,5-epoxide has been isolated and identified as a precursor of the BaP-4,5-dihydrodiol. Other studies indicate that epoxides are the precursors of the 7,8-dihydrodiol and 9,10-dihydrodiol as well.

Since the resonance properties of POM make ring openings difficult, enzymatic attack in the microsomes functions to open double bonds and add an oxygen-containing moiety, such as a hydroxyl group, to give it more solubility in aqueous media (e.g., urine) and thus facilitate removal from the body. In the formation of metabolic intermediates by oxidation mechanisms,

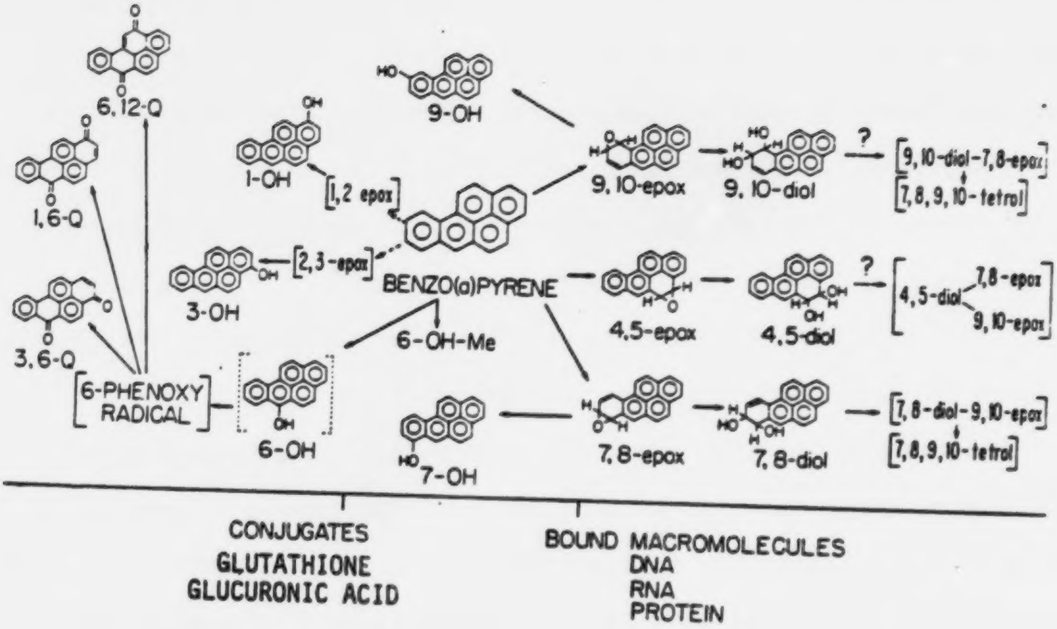

6,12-Q
1,6-Q
3,6-Q
9-OH
1-OH
[1,2 epox]
3-OH
[2,3-epox]
BENZO(a)PYRENE
6-OH-Me
6-PHENOXY RADICAL
6-OH
7-OH
9,10-epox
9,10-diol
?
[9,10-diol-7,8-epox]
[7,8,9,10-tetrol]
4,5-epox
4,5-diol
?
4,5-diol
7,8-epox
9,10-epox
7,8-epox
7,8-diol
[7,8-diol-9,10-epox]
[7,8,9,10-tetrol]
CONJUGATES
GLUTATHIONE
GLUCURONIC ACID
BOUND MACROMOLECULES
DNA
RNA
PROTEIN

Figure 6-8. Oxidative metabolites of benzo[a]pyrene.

relatively stable POM are converted to unstable products (i.e., epoxides). Thus, nucleophilic attack of this reactive intermediate, through the formation of a transient carbonium ion, would be greatly enhanced. Arylations of this type are common to many classes of carcinogenic chemicals. Therefore, the microsomal cytochrome P-450-containing MFO system and epoxide hydrase play a critical role in both the metabolic activation and detoxification of many POM.

Various forms of liver microsomal cytochrome P-450 can be isolated from animals treated with different enzyme inducers.[59,60,61,62] Moreover, the metabolite profiles of BaP can be qualitatively altered depending on the type of cytochrome P-450 present in the incubation mixture.[40,63] This observation has important implications in considering the carcinogenic action of certain POM toward tissues from animals of different species, sex, age, nutritional status, and exposure to enzyme-inducing chemicals. Limited evidence is also available indicating that multiple forms of epoxide hydrase exist among animal species, which may also influence the pattern of POM metabolism with respect to carcinogenic bioactivation.[62]

The metabolic fate of most POM has not been studied as extensively as that of BaP. Nevertheless, several generalizations are evident which apply to most unsubstituted and alkyl-substituted polycyclics. To a limited extent, direct attack on saturated carbon atoms may occur to form, sequentially, alcohols, ketones, aldehydes, and carboxylic acids.[100] More commonly, metabolic attack on one or more of the aromatic double bonds (K-region and non-K-region) leads to the spontaneous formation of phenols or of isomeric dihydrodiols by the intermediate formation of reactive epoxides.[100,101] Dihydrodiols are further metabolized by the microsomal monooxygenases to yield diol-epoxides, compounds which are implicated as ultimate biologically reactive intermediates. Removal of activated intermediates by conjugation with glutathione or glucuronic acid, or by further metabolism to tetrahydrotetrols, is a key step in protecting the organism from toxic interaction with cell macromolecules.[34,102]

With unsubstituted POM, such as benz[a]anthracene and DBahA, oxidation occurs with rat liver homogenates to produce phenols and dihydrodiols.[34,103-105,530] Both K-region and non-K-region (see Section 6.4.2) oxidative metabolites are formed, which may subsequently be conjugated with glutathione and excreted in the urine as mercapturic acids. In addition, hydration of the epoxide intermediates formed from these compounds may yield dihydrodiols. Benz[a]anthracene is metabolized by rat liver microsomes in the presence of epoxide hydrase primarily to its 5,6- and 8,9-dihydrodiols.[530] Small amounts of the 3,4- and 10,11-dihydrodiols are detected, but only trace quantities of phenols are formed. It is the 3,4-dihydrodiol of benz[a]anthracene that is a proximate carcinogenic metabolite, as predicted by the bay-region theory (see Section 6.4.2).[531] The major metabolite of DBahA formed by rat liver microsomes is the 3,4-dihydrodiol, which is also the immediate precursor of the carcinogenic diol-epoxide of DBahA as predicted by the bay region theory (see Section 6.4.2, Section 6.4.3.4, and Section 6.4.5).[532] Evidence indicates that K-region epoxides are better substrates for the formation of glutathione conjugates than non-K-region epoxides.

Among the simpler unsubstituted POM's, such as phenanthrene, no evidence for the formation of a K-region phenol or a conjugate thereof can be detected.[100]

The major phenanthrene metabolite is a K-region dihydrodiol which may also be excreted as a conjugate with glucuronic or sulfuric acid.

In contrast to the unsubstituted compounds, POM-bearing alkyl substitutes (e.g., MCA, DMBA) are primarily hydroxylated at the alkyl side chain. The metabolism of DMBA has been carefully studied using rat liver homogenates.[106,107] These studies clearly showed that the main products of DMBA metabolism are the isomeric 7-hydroxymethyl-12-methylbenz[a]anthracene and 12-hydroxymethyl-7-methylbenzanthracene. In addition, DMBA and its hydroxymethyl derivatives are metabolized into 3,4- and 8,9-dihydrodiols (non K-region). In contrast, a K-region 5,6-epoxide is also formed, but instead of being hydrated to the dihydrodiol, it is conjugated with glutathione.[106] Although all of the polar DMBA metabolites have not yet been identified, no derivative has thus far been detected which involves enzymic oxidation at both the 5,6- and 8,9-bonds in the same molecule. Recent studies have implied that metabolic activation of DMBA for binding with DNA occurs through the generation of a diol epoxide in the 1,2,3,4-ring.[108] Ring hydroxylation at the 3- and 4-positions of DMBA (M-region) had previously been shown to occur.[109] Thus, evidence for the "bay region" theory being of general applicability is increasing.

Metabolism of the carcinogens 7- and 12-methylbenz[a]anthracene is qualitatively similar to DMBA.[109] The products formed by rat liver homogenates include: dihydrodiols at the 5,6-(K-region) and 8,9-(non-K-region) positions; hydroxymethyl derivatives; various phenols; and glutathione conjugates at the K-region. Figure 6-9 presents a probable scheme for the metabolism of 7-methylbenz[a]anthracene which would also apply to 12-methylbenz[a]anthracene.

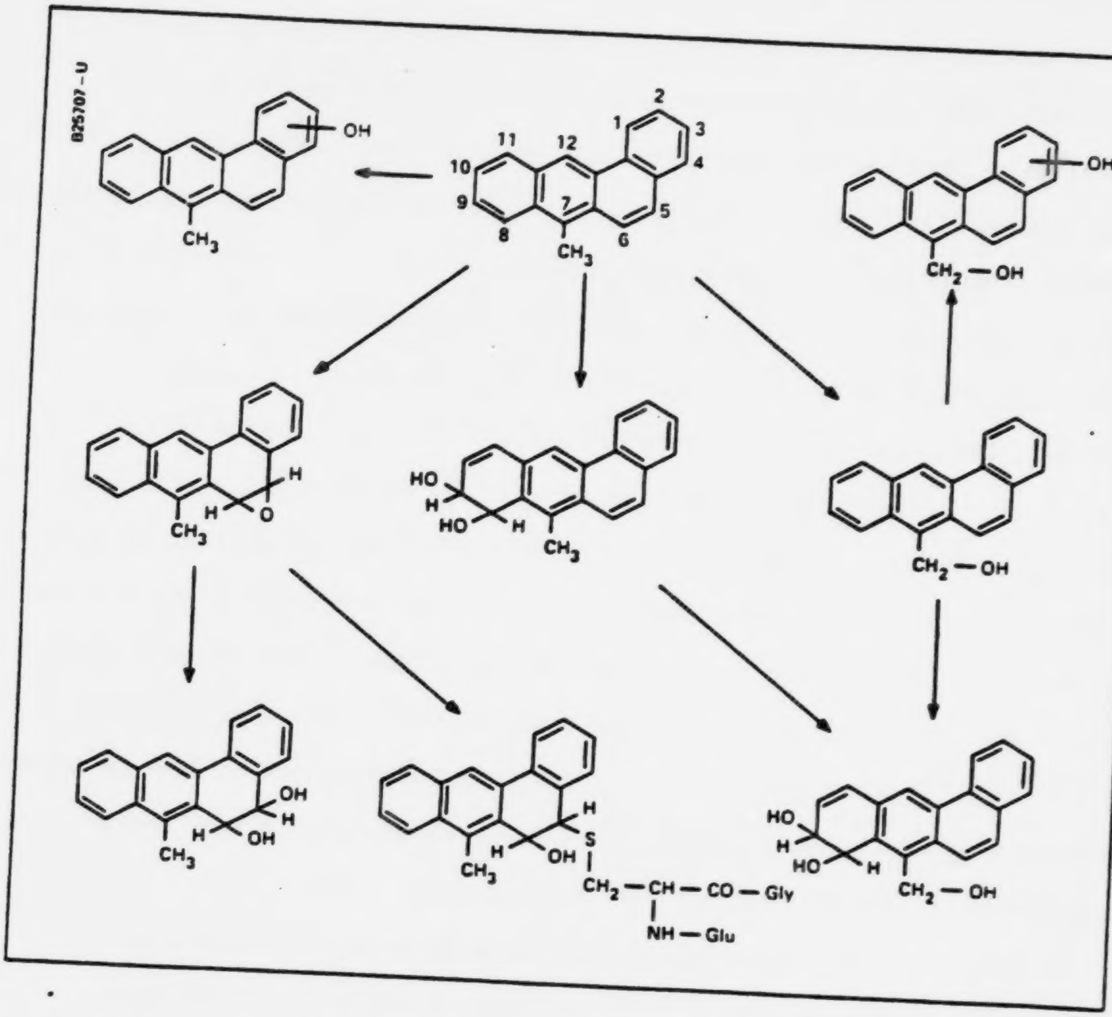

B25707-U
OH
CH₃
1
2
3
4
5
6
7
8
9
10
11
12
CH₃
OH
CH₂—OH
H
H
O
CH₃
HO
H
HO
H
CH₃
CH₂—OH
OH
H
H
OH
CH₃
H
S
H
OH
CH₃
CH₂—CH—CO—Gly
NH—Glu
HO
H
HO
H
CH₂—OH

Figure 6-9. Probable metabolic pathways of 7-methylbenz[a]anthracene.[109]

The metabolites of MCA formed by rat liver homogenates, liver microsomes, or a highly purified cytochrome P-448-dependent monooxygenase system with epoxide hydrase are all similar.[53,110] Formation of 1-hydroxy MCA and 2-hydroxy MCA predominates. Smaller amounts of the K-region trans 11,12-dihydrodiol and a glutathione conjugate derived therefrom are formed from MCA, as well as trans 1,2-dihydroxy MCA and several phenols. The two major dihydrodiols which are produced by the action of epoxide hydrase on (±) 1-hydroxy MCA are the diastereometrically related trans 9,10-dihydrodiol. These results led Thakker and coworkers[53] to postulate that the ultimate carcinogenic metabolite of MCA may be the "bay region" diol epoxides (9,10-diol-7,8-epoxides) derived from 1-hydroxy MCA.

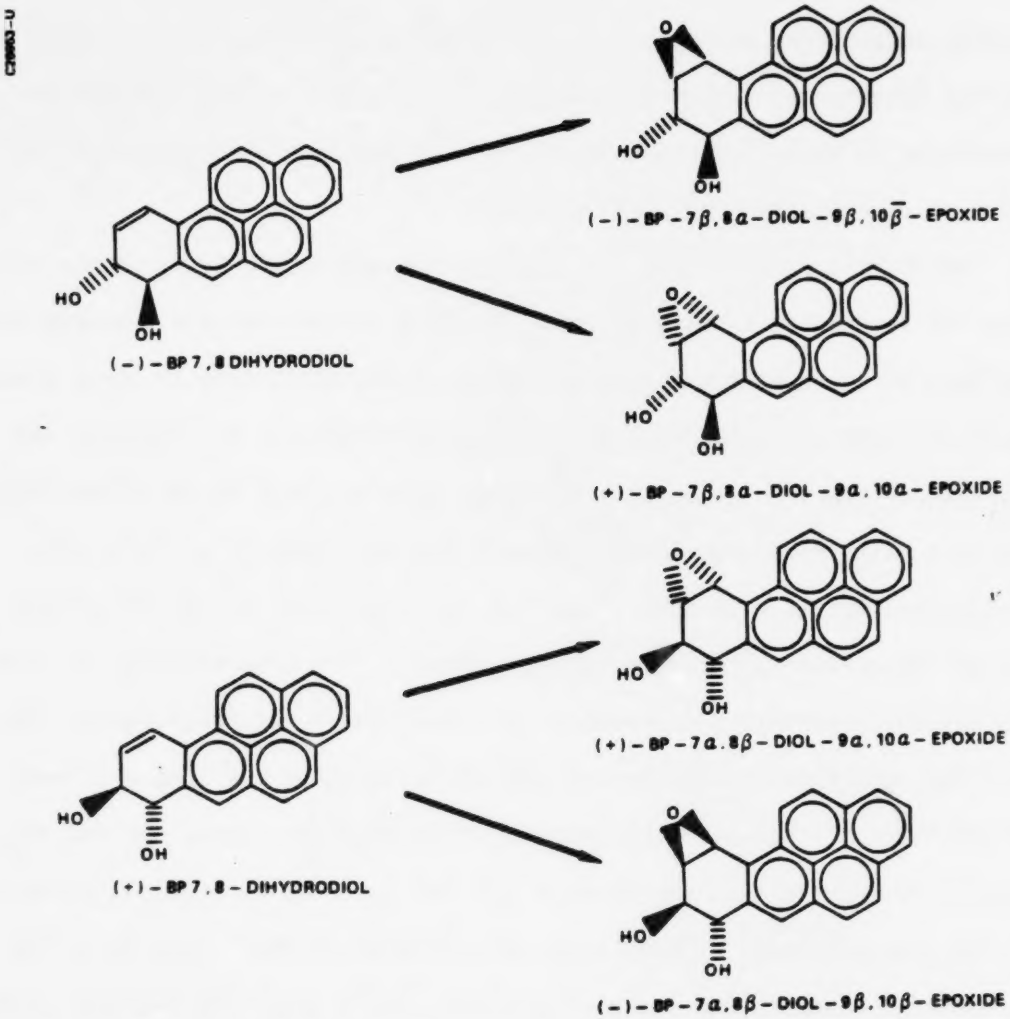
An important consideration in evaluating the health hazards of POM is whether metabolism in rat liver preparations is indicative of the pattern of POM metabolism in more likely target organs (e.g., lung) (see Section 6.2.3). Moreover, it is vital to determine whether metabolism in rodents mimics metabolism in man (see Section 6.2.4). In this regard, it has been shown that the metabolism of benz[a]anthracene, 7-methylbenz[a]anthracene, and BaP was qualitatively similar in rat liver and lung.[111,112] Both K-region and non K-region dihydrodiols were detected, which almost certainly arose from epoxide precursors.

6.2.2 Metabolic Bioactivation of Benzo[a]pyrene

The role of metabolic biotransformation in conferring carcinogenic activity for POM has been clearly established using BaP as a model compound. While it is known that BaP is metabolized by the MFO system to various arene oxides, phenols, dihydrodiols, and diol epoxides, it is the latter type of intermediate which has the greatest toxicologic significance.[54,58,64-70] The BaP-7,8-diol-9,10-epoxides are derived from BaP-7,8-dihydrodiol and exist as a pair of diastereomers in which the 7-hydroxyl group is either cis or trans to the 9,10-epoxide moiety (Figure 6-10). Each of the diastereomeric BaP-7,8-diol-9,10-epoxides can be further resolved into a pair of optical enantiomers, the relative amounts of which depend on the stereoselectivity of the final oxygenation step catalyzed by the MFO system.[47,71]

In liver microsomes from rats pretreated with MCA, BaP is stereoselectively metabolized to the (-)-enantiomer of BaP-7,8-dihydrodiol and to (+)-BaP-7β,8α-diol-9α,10α-epoxide. These metabolites possess high mutagenic and carcinogenic activity compared to the parent compound and other BaP metabolites,[67,68,72,73] and are believed to be proximate and ultimate carcinogenic intermediates of BaP, respectively (see Section 6.4.2 and Section 6.4.3.1). In particular, the (+)-trans-BaP-7,8-diol-9,10-epoxide was shown to be the most carcinogenic of the four BaP-diol epoxide isomers in newborn mice,[73] the most mutagenic of the four isomers in Chinese hamster V79 cells,[72] and is the isomer which stereoselectively binds to the exocyclic amino group of guanine in double-stranded calf thymus DNA.[74] Therefore, evidence is very strong to indicate that (+)-BaP-7β,8α-diol-9α,10α-epoxide is the major ultimate carcinogenic metabolite of BaP. Furthermore, Buening and coworkers[73] have suggested that (+)-BaP-7β,8α-diol-9α,10α-epoxide may be the most potent compound ever tested for the induction of pulmonary tumors in mice.

Factors affecting the direction of BaP metabolism have obvious toxicologic significance in determining its carcinogenic activity in various tissues, individuals, and species. In this regard, Deutsch and coworkers[71]



C29863-U
HO
OH
(−) − BP 7, 8 DIHYDRODIOL
O
HO
OH
(−) − BP − 7β, 8α − DIOL − 9β, 10β − EPOXIDE
O
HO
OH
(+) − BP − 7β, 8α − DIOL − 9α, 10α − EPOXIDE
HO
OH
(+) − BP 7, 8 − DIHYDRODIOL
O
HO
OH
(+) − BP − 7α, 8β − DIOL − 9α, 10α − EPOXIDE
O
HO
OH
(−) − BP − 7α, 8β − DIOL − 9β, 10β − EPOXIDE

Figure 6-10. Stereochemical course of metabolism of (+) and (-)-BaP 7,8-dihydrodiol.

and Thakker and coworkers[47] have shown how the various forms of cytochrome P-450 from rabbit and rat liver microsomes influence the regiospecific and stereoselective metabolism of BaP. Different forms of cytochrome P-450 had marked effects on the conversion of BaP and its (-)-7,8-dihydrodiol to diol epoxide metabolites which bind to DNA. The balance of activities among the various cytochromes vis-à-vis exposure to microsomal enzyme inducers was thus considered to be an important determinant of carcinogenic susceptibility.

6.2.3 Tissue-Specific Metabolism of POM

Metabolism studies have historically focused on the liver as the principal site for xenobiotic transformation. Recently, a considerable research effort has been directed at the characterization of POM metabolism in those tissues which are most susceptible to malignant transformation, particularly the respiratory tract. The objective of this work has been to correlate tissue-specific patterns of POM metabolism with the carcinogenic activity of a particular substance in that organ. In addition, the actions of various enzyme inducers in altering metabolic pathways have been examined to determine how various xenobiotics can modify the bioactivation of carcinogenic POM.

The cytochrome P-450 content and MFO activity of the lung are lower than in the liver.[75] However, the activity of conjugating enzyme systems and epoxide hydrase in the lung is very low, and thus may be critical in determining susceptibility of this organ to carcinogenic POM. Studies on the metabolism of BaP in the isolated perfused rabbit lung revealed that arene oxide intermediates formed by the oxidative metabolism of BaP were further enzymatically transformed by epoxide hydrase and glutathione S-transferases.

The removal of arene oxides by hydration and conjugation, however, proceeded at a rate which was an order of magnitude less than the overall rate of metabolism.

Pretreatment of rabbits with MCA, a microsomal enzyme inducer, had no effect on the metabolism of BaP by microsomal preparations, nor did it increase cytochrome P-448 in pulmonary microsomes. In the isolated perfused rabbit lung, pretreatment with MCA increased only the epoxide hydrase activity, and consequently the rate of production of dihydrodiol metabolites.

In contrast to the metabolic activity of the rabbit lung toward POM, the lungs of rats and mice respond to pretreatment by enzyme inducers with a 4- to 25-fold increase in BaP hydroxylase activity.[75] Recent studies by Vahakangas and coworkers[76,77] compared the effects of pretreatment with MCA or phenobarbitone on the metabolism of BaP in isolated perfused rat lungs. Pretreatment with MCA enhanced both the rate of BaP hydroxylation and the activity of epoxide hydrase. This was evidenced by an increase in measured rates of BaP hydroxylase enzyme activity and enhanced production of dihydrodiol metabolites. In addition, MCA pretreatment stimulated conjugation reactions involving BaP metabolites. Accompanying the enhancement of BaP metabolism by MCA pretreatment was an increase in covalent binding of BaP metabolites to lung tissue. A similar increase in covalent binding was produced by exposing rats to cigarette smoke, and thus implicated enzyme induction by environmental agents as being toxicologically significant in the lung.

Although pretreatment of rats with phenobarbitone slightly increased the rate of BaP hydroxylation in rat liver and kidney (Table 6-10), it had the opposite effect in the rat lung.[76,77] Moreover, the activities of epoxide hydrase and the conjugating enzymes in rat lung were not enhanced by

Table 6-10. BaP-HYDROXYLASE ACTIVITY IN DIFFERENT ORGANS OF THE RAT AFTER METHYLCHOLANTHRENE AND PHENOBARBITONE PRETREATMENT[77]

Pretreatment	BaP-hydroxylase (fluorescence units/g x min.)		
	Perfused lung	Liver	Kidney
3-Methylcholanthrene	388 ± 55*	26982 ± 4957*	258 ± 85*
Control	114 ± 31	5527 ± 1392	38 ± 13
Phenobarbitone	54 ± 9	4872 ± 2048	21 ± 4
Control	82 ± 19	3674 ± 909	11 ± 5

* Significantly different from control ($P<0.05$) by Student's t-test.

The values represent means ± S.E. of 6 animals in each group. The livers and kidneys were removed from the same animals as the lungs for perfusion.

phenobarbitone pretreatment. Previous investigators, however, have reported epoxide hydrase to be inducible by phenobarbitone in the lungs.[54]

Since the tracheo-bronchial mucosa is the respiratory target tissue for POM-induced cancer in both animals and man, a number of studies have focused on the metabolism of BaP in rodent trachea. The importance of enzyme inducers in determining the fate and toxicologic significance of POM in the trachea was illustrated in studies by Simberg and Votila.[78] They reported that rats receiving daily 1-hour exposures to cigarette smoke for one or 10 days displayed an increase (290 to 320 percent) in MFO activity in the trachea. Furthermore, a two-fold increase in covalent binding of BaP metabolites to nucleic acid and protein fractions of the trachea occurred 12 hours after a single cigarette smoke exposure. No changes were detected in the activity of epoxide hydrase in the trachea, and only a slight increase in UDP-glucuronosyltransferase in the trachea and lungs resulted from repeated cigarette smoke exposures. Enhancement of MFO activity by cigarette smoke in rat lungs was nearly double the increase observed in the trachea.

Moore and Cohen[79] conducted an extensive study on the metabolism of BaP and its major metabolites in cultured trachea of rats and hamsters. The predominant ethyl acetate-soluble (Phase 1) metabolites of BaP in the trachea of both species were the 9,10-dihydrodiol and 7,8-dihydrodiol (Table 6-11). Smaller amounts of the 4,5-dihydrodiol, monohydroxybenzo[a]pyrenes, benzo[a]-pyren-3-yl hydrogen sulfate, and tetrols were also found. Much of the 4,5-dihydrodiol and monohydroxybenzo[a]pyrenes produced from BaP was rapidly conjugated and thus removed to the water-soluble metabolite fraction. This explains previous observations that only small amounts of 4,5-dihydrodiol and monohydroxybenzo[a]pyrenes can be found as organic solvent-soluble metabolites

Table 6-11. SUMMARY OF MAJOR ORGANIC SOLVENT-SOLUBLE AND WATER-SOLUBLE METABOLITES FOLLOWING THE SHORT-TERM TRACHEAL ORGAN CULTURE OF BP OR ITS METABOLITES.[a] 77

Compound metabolized	Species	Ethyl acetate-soluble metabolites	Water-soluble metabolites
BP	Hamster	9,10-Dihydrodiol,[b] 7,8-dihydrodiol, 4,5-dihydrodiol, monohydroxybenzo(a)pyrenes, and benzo(a)pyrene-3-yl hydrogen sulfate[b]	Glucuronide conjugates of 3-hydroxybenzo(a)pyrene,[b] 4,5-dihydrodiol, 7,8-dihydrodiol,[b] 9,10-dihydroxybenzo(a)pyrene[b]
3-OH-BP	Rat and hamster	Benzo(a)pyren-3-yl hydrogen sulfate[b]	Glucuronide conjugate of 3-hydroxybenzo(a)pyrene
9,10-Dihydrodiol	Rat	9,10-Dihydroxybenzo(a)pyrene	Glucuronide conjugate of 9,10-dihydroxybenzo(a)pyrene[b]
9,10-Dihydrodiol	Hamster	7,8,9,10-Tetrahydrotetrol[b]	Glucuronide conjugate of 9,10-dihydroxybenzo(a)pyrene
7,8-Dihydrodiol	Rat and hamster	7,8,9,10-Tetrahydrotetrol[b]	Glucuronide conjugate of 7,8-dihydrodiol
4,5-Dihydrodiol	Hamster	Unidentified metabolite (possibly tetrahydrotetrol)	Glucuronide conjugate of 4,5-dihydrodiol

[a] Metabolites were identified on the basis of cochromatography with standard metabolites, by TLC in 1 or more solvent systems.

[b] Water-soluble metabolites were identified following hydrolysis with either Ketodase (for glucuronide conjugates) or arylsulfatase with D-saccharic acid 1,4-lactone (for sulfate conjugates) and then examination of the ethyl acetate-soluble products by TLC.

[c] These metabolites were also identified on the basis of fluorescence excitation and emission spectra similar to the standards.

in cultured human and rodent respiratory tissue.[80] Metabolism of the 7,8-dihydrodiol and 9,10-dihydrodiol yielded the corresponding tetrahydrotetrols via their respective diol-epoxides. In addition, a major metabolic pathway for the 7,8-dihydrodiol was to the water-soluble glucuronide conjugate, whereas almost no glucuronide conjugate was formed from the 9,10-dihydrodiol.

The presumed importance of the BaP 7,8-dihydrodiol as a proximate carcinogen is a primary reason why tissue-specific patterns of metabolism may ultimately determine target organ susceptibility to BaP-induced cancer. As suggested by the work of Moore and Cohen,[79] an alteration by environmental or genetic factors of the activities of the conjugating enzymes can affect the levels of 7,8-dihydrodiol, and consequently the formation of reactive diol epoxide intermediates.

Investigation of the metabolism of BaP in rodent trachea has been extended to include cultured human bronchus as well. In a series of experiments it was shown that human bronchial mucosa contains MFO activity that is readily inducible by pretreatment with POM such as benz[a]anthracene and BaP, although large interindividual variations apparently existed.[80] On the other hand, the activity of epoxide hydrase was not affected by benz[a]anthracene. Enhanced MFO activity in human bronchial mucosa was reflected in an observed increase in BaP binding to DNA following pretreatment with BaP or benz[a]anthracene. One of the major metabolites of BaP that binds to DNA in human bronchus was tentatively identified as a diol epoxide enzymatically derived from the (-)-trans-7,8-dihydrodiol of BaP. Therefore, these results in human respiratory tissue support the data from similar studies in animals regarding the importance of tissue-specific MFO activity in the bioactivation of POM to carcinogenic intermediates which bind to DNA. More important, these studies strengthen the argument that certain POM can induce bronchial cancer in humans.

Among the non-hepatic tissues investigated for their capability to metabolize POM, the intestinal mucosa has received considerable attention. Although the MFO activity of the intestinal tract is low when compared to the liver, it is nevertheless a site of POM-induced cancer in animals. Bioactivation of POM by intestinal cells would, therefore, be expected to occur in all susceptible species. This contention was recently confirmed by studies showing that susceptibility to BaP-induced forestomach tumors in mice was correlated with MFO activity in the same tissue.[81]

Another group of workers[82] has examined BaP metabolism by microsomes and isolated epithelial cells from rat small intestine. Their results agreed with those obtained by previous investigators showing that cytochrome P-450 content and MFO activity are normally low in the intestinal epithelium. However, MCA displayed a marked stimulatory effect on both cytochrome P-450 content and MFO activity. In contrast to the situation with rat liver, neither phenobarbital nor pregnenolone-16a-carbonitrile caused substantial increases in MFO activity. Their results further demonstrated that the epoxide hydrase activity of rat small intestine was very low and non-inducible. This was evidenced by the fact that relatively little dihydrodiol formation resulted from the incubation of BaP with microsomes from intestinal cells as compared to liver cells from MCA-pretreated rats (Table 6-12).

Fang and Strobel[83,84] have recently demonstrated that rat colon mucosa possesses a microsomal drug-metabolizing enzyme system. They evaluated the ability of colon 9000 x g supernatant (S9) fractions to activate BaP to

Table 6-12. COMPARISON OF BENZO[a]PYRENE METABOLITE PATTERNS IN LIVER AND INTESTINAL MICROSOMES FROM MC-TREATED RATS.[82]

Benzo[a]pyrene metabolites	Liver	Small intestine
	% of total metabolites	
More polar than 9,10-dihydrodiol	1.5	7.0
9,10-dihydrodiol	18.0	0.5
4,5-dihydrodiol	11.5	1.5
7,8-dihydrodiol	10.5	3.5
1,6-quinone	10.0	11.0
3,6-quinone	12.0	12.5
6,12-quinone	3.5	7.5
4,5-oxide	--	14.5
9-hydroxy	6.5	14.0
3-hydroxy	14.0	17.0
Other	12.5	11.5
	100%	100%

metabolites which were mutagenic in the Ames Salmonella test system. Activation of BaP was examined using colon S9 preparation from rats treated with various enzyme inducers, and the results compared with those obtained using corresponding liver S9 preparations. Their results, summarized in Table 6-13, indicated that an approximate doubling of the mutation rate occurred with addition to the test system of colon or liver S9 preparations from uninduced rats. Pretreatment of rats with β-naphthoflavone induced a four-fold increase in the activation of BaP by the colon, whereas phenobarbital/hydrocortisone and Aroclor 1254 had no effect. These results suggest a role for POM bioactivation by the MFO system in colon carcinogenesis, a theory supported by the observation that cultured human colon can activate carcinogens leading to covalent protein binding.[85] Furthermore, these results indicate that dietary constituents having the ability to induce or inhibit MFO activity in the gastrointestinal tract may have a strong influence on individual susceptibility to colon cancer.

In any mammalian tissue, differences in the quantity and/or structure of reactive intermediates of POM formed by the MFO system are clearly reflected in the degree of susceptibility to neoplastic transformation. Genetic factors affecting the delicate balance of activating and detoxifying enzymes produce differences in the metabolism and binding to DNA of carcinogenic POM.[86,87] These factors, in combination with diet and simultaneous exposure to non-carcinogenic chemicals, must be considered in evaluating the carcinogenic potential of POM and in explaining observed differences in response among species and target organs. In addition, the sex-dependent regulation of POM metabolism has been demonstrated for certain rat tissues,[88] and thus adds still another dimension to the interpretation of experimental results.

Table 6-13. ACTIVATION OF BENZO[a]PYRENE BY COLON AND LIVER S9 FRACTIONS[a]

Pretreatment group	Revertants/mg S9 protein			
	25 μg		50 μg	
	Colon	Liver	Colon	Liver
Control	63 ± 2.7[b]	103 ± 6.5	65 ± 8.6	111 ± 6.7
Phenobarbital/hydrocortisone	65 ± 2.5	75 ± 1.7	73 ± 8.7	61 ± 3.4
β-Naphthoflavone	239 ± 13.5[c]	476 ± 14.2[b]	233 ± 9.3[b]	596 ± 29.8[b]
Aroclor 1254	72 ± 2.2	497 ± 45.0[b]	85 ± 1.2	186 ± 6.7[b]

[a] Liver and colon 9000 x g supernatant fractions were prepared from control rats or rats pretreated with phenobarbital/hydrocortisone, β-naphthoflavone, or Aroclor 1254. These fractions were tested in an Ames mutagenesis assay system with *S. typhimurium* TA100 for their ability to activate the precarcinogen benzo(a)pyrene. The results are reported as revertants/mg S9 protein. Each value has been corrected for spontaneous revertants occurring in the absence of either the precarcinogen or the S9 fraction.

[b] Mean ± S.E. for at least 3 separate determinations.

[c] Significantly increased over control group, $p<0.01$.

6.2.4 Comparative Metabolism of POM in Animals and Man

An important consideration in evaluating the health hazards of POM is whether metabolism in various animal tissues and species is indicative of the pattern of POM metabolism in the target organs of humans. Moreover, it is essential to determine whether differences occur in the metabolism of POM by: (a) different tissues in the same animal; and (b) different animals of the same species.

Numerous studies have shown that qualitative and quantitative differences exist in the metabolism of BaP by different tissues and animal species.[34,89-93] For the most part, however, interspecies extrapolations of qualitative patterns of POM metabolism appears to be a valid practice. On the other hand, marked differences in patterns of tissue-specific metabolism may prevent the reliable extrapolation of data from hepatic to extrahepatic (i.e., target organ) tissues. These differences may also exist in human tissues, possibly due to different levels of drug-metabolizing enzymes present, and differences in responsiveness to enzyme-inducing agents in specific organs.[94]

Nevertheless, it has been adequately demonstrated that human cells possess the mechanisms to bioactivate POM to carcinogenic intermediates. When added to cultures of human skin fibroblasts, BaP caused an induction of MFO activity, increased cellular proliferation, and produced DNA damage.[95] On the other hand, MCA was relatively ineffective, while DMBA was capable of inducing MFO activity and cell proliferation, but did not produce DNA damage. In addition, noncarcinogenic POM such as anthracene and phenanthrene had no effect on the human cells. The fact that neither MCA nor DMBA could be detected in the cell nucleus implies that in human cells the necessary metabolic sequences for bioactivation of POM carcinogens may not operate in all tissues and for all such compounds in the class. This may explain the failure to induce neoplastic or morphologic transformation with POM treatment *in vitro* of randomly proliferating human cell populations.

Freudenthal and coworkers[96] recently examined the metabolism of BaP by lung microsomes isolated from the rat, rhesus monkey, and man. Metabolite profiles obtained by high pressure liquid chromatography are shown in Figure 6-11. Their results confirmed previous observations regarding the existence of considerable individual variation in BaP metabolism among samples from the same species. In addition, it was apparent that qualitative and quantitative inter-species variation also existed (Table 6-14). Nevertheless, the qualitative differences between man and the other animal species were by no means dramatic, and probably do not compromise the validity of extrapolations concerning POM metabolism.

In other investigations of BaP metabolism by human lung microsomal fractions, it was noted that a higher percentage of dihydrodiol metabolites is formed in the lung of humans as compared to the rat.[99] This result may reflect differences in relative levels of epoxide hydrase in human versus rat lung, and can be expected to influence carcinogenic susceptibility. The authors felt that the relative insensitivity of the rat lung to POM-induced cancer supports this conclusion. However, large variations in metabolite profiles formed by the 15 human specimens employed in this study indicate that generalizations cannot yet be stated regarding the metabolic activity of human lung.

The metabolite pattern obtained for BaP in human monocytes is similar to that obtained with human liver microsomes[45] and human lymphocytes.[97]

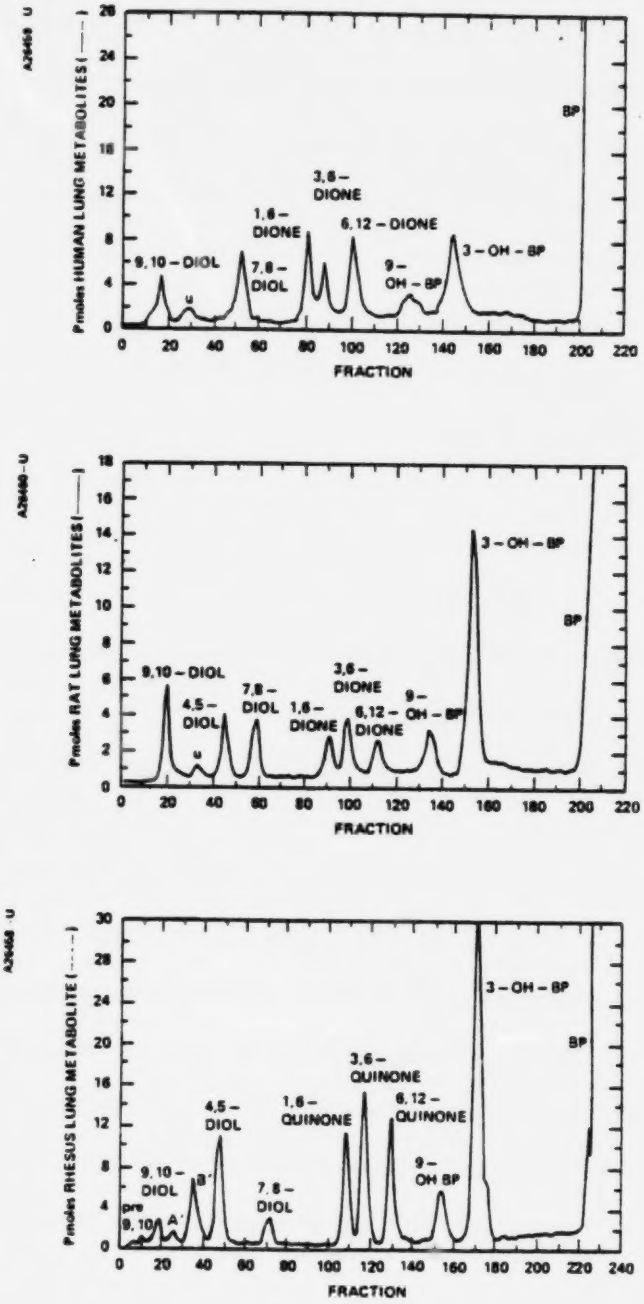


Figure 6-11. Comparative metabolism of benzo[a]pyrene by lung microsomes from rat, rhesus monkey, and human[96]

Table 6-14. METABOLITE PERCENTAGES OF BP METABOLITES FROM RAT, RHESUS, AND HUMAN LUNG MICROSOMAL ASSAYS[96]

Metabolite	Metabolite percentages (pmoles metabolite/pmoles total metabolites x100)									
	Rat[a]			Rhesus[b]			Man[b,c]			
	1	2	3	1	2	3	1	2	3	4
Pre-9,10					3.0	5.3				
9,10-Diol	9.7	6.3	9.6	2.7	4.6	2.6		7.1	6.0	
A				1.5						
U (B)	4.4	3.4	2.9	6.9		7.7	8.9	3.9	7.5	30.0
4,5-Diol	8.3	9.2	8.3	9.0	9.2	7.7	4.1			
7,8-Diol	5.3	5.2	8.0	4.2	8.6	5.1		15.0	13.3	9.9
1,6-Dione	4.4	7.5	8.3	11.4	14.8	12.8	24.9	11.6	12.6	4.4
3,6-Dione	7.8	8.0	9.9	14.5	16.0	20.5	22.5	13.8	19.2	8.5
6,12-Dione	6.8	8.6	8.6	11.8	8.0	15.3	22.5	18.3	27.4	15.7
9-OH	12.6	11.5	3.5	7.3			5.7	6.2		8.5
3-OH	40.8	40.2	41.1	30.8	35.9	23.1	11.4	24.0	13.9	22.9

[a] Lungs of 5 rats pooled for each group.

[b] Determinations made on lung samples from separate individuals.

[c] With the exception of subject 4, activity determinations were made using microsomes which had been stored at -84°C.

[d] The structural characteristics of unknown, U, may differ between species.

However, in cultured human bronchus (24 hours) and pulmonary alveolar macrophages an absence of phenols (i.e., 3-hydroxy-BaP) and paucity of quinones was observed.[98] Instead, a relative abundance of the trans-7,8-dihydrodiol metabolite of BaP was demonstrated. This result confirms the work of others[99] and is noteworthy in light of the possibility that the 7,8-dihydrodiol is capable of further oxidative metabolism to an ultimate carcinogenic form of BaP. It is not known whether a longer incubation period would have changed the pattern of metabolite formation.

6.2.5 Actions of Enzyme Inducers

The metabolism of most lipophilic xenobiotics is catalyzed by the microsomal mixed-function oxidase system.[32,113] One of the oxidases in this system, designated aryl hydrocarbon hydroxylase (AHH), is responsible for both the detoxification and metabolic activation of POM. AHH is the term used for the cytochrome P-450-dependent monooxygenase systems that metabolize BaP to fluorescent phenols. The activity of AHH can be induced (i.e., enhanced) by substrate and non-substrate xenobiotics, either in vivo or in cultured mammalian cells.[61] This enzyme can be found in 90 percent of the tissues examined in rats, mice, hamsters, and monkeys.[113] These include liver, lung, testis, muscle, bone marrow, skin, brain, intestine, placenta, and cultured mammary gland, lymphocytes, leukocytes, and monocytes.[36-42,113] The ultimate cytotoxicity and/or carcinogenicity of POM may in large part be determined by the level of AHH activity in the organ of exposure. The important role of AHH in the susceptibility to POM-induced damage results from the fact that POM must usually be metabolized to reactive intermediates before toxicity can be expressed. In this regard, it has been shown that cell lines which were highly resistant to the toxic effects of BaP were also lacking in AHH activity.[113]

For more than 20 years it has been known that POM's such as BaP, DBahA, MCA, and chrysene are potent inducers of AHH in liver microsomes.[61] Thus it is not surprising that cigarette smoke has a marked stimulatory effect on AHH in lung, liver, intestine, and human placenta. Intragastric administration of MCA increases AHH activity of rat liver, kidney, and intestinal mucosa,[20] and intraperitoneal injection increases AHH activity in rabbit bone marrow.[38] In cultured hamster fetus cells, Nebert and Gelboin[114] established that AHH activity was readily induced by carcinogenic and noncarcinogenic POM. Maximum enzyme induction was generally produced with a POM concentration of 10 μM in the culture medium. AHH inducibility in animals is under genetic control, and it can be shown that noninducible strains of mice are also resistant to the carcinogenic effect of subcutaneously injected MCA.[115]

Kellerman and coworkers[116,117] have used human lymphocytes to demonstrate that AHH inducibility by MCA may also be under genetic control in humans, and further that the extent of AHH induction may correlate with susceptibility to chemical carcinogenesis. These investigators observed a virtual absence of lung cancer among patients having little capacity for AHH induction. Therefore, it may be postulated that genetic heterogeneity with regard to microsomal enzyme induction might account for the apparently inherited susceptibility (i.e., familial clustering) for bronchogenic carcinoma. However, difficulty in reproducibility of the assay for AHH inducibility in lymphocytes and monocytes, as well as large temporal and inter-individual variability, have prevented an evaluation of its genetic control in humans.[36,118-121] At the present time, attempts to confirm several aspects of Kellerman's studies have been unsuccessful.

Paigen and coworkers[121] have examined the question of genetic susceptibility to cancer and concluded that epidemiologic evidence supports this hypothesis. Moreover, they were able to show that AHH inducibility in lymphocytes segregates in the human population as a genetic trait. However, their studies failed to find a correlation between this inducibility and presumed cancer susceptibility, either among healthy relatives of cancer patients or in patients who had their cancer surgically removed. It is noteworthy that previous investigations on AHH inducibility were conducted in persons with active cancer.

Recent studies with other human tissues (liver and placenta) have provided important new data concerning the carcinogen-metabolizing capacity of man and its implications for cancer susceptibility. Conney and coworkers[122] examined individual differences in the metabolism of drugs and carcinogens in human tissues, and have identified drugs which may serve as model substrates to provide an indirect index of carcinogen metabolism for man. The rates of antipyrene, hexobarbital, and zoxazolamine hydroxylation in human autopsy livers were highly, but not perfectly, correlated with the rates of BaP metabolism. In human placenta, an almost perfect correlation was found between zoxazolamine hydroxylase activity and BaP hydroxylase activity.[123] Thus, metabolism of BaP and zoxazolamine by human placenta occurs by the same enzyme system(s) or by different enzyme systems under the same regulatory control.[124] BaP and zoxazolamine hydroxylase activities were also shown to be significantly enhanced in placentas obtained from women who smoked cigarettes. The lack of perfect correlations for the hepatic metabolism of BaP and certain drugs in many subjects indicated the presence of several monooxygenases in human liver which catalyze the oxidative metabolism of these compounds. Furthermore, large inter-individual differences exist in the capacity of humans to metabolize foreign chemicals both *in vitro* and *in vivo*. Further studies showed that 7,8-benzoflavone markedly stimulated the hydroxylation of BaP, antipyrene, and zoxazolamine in human liver samples, but with a wide variation in magnitude among different samples. These results suggested the presence of multiple monooxygenases or cytochrome P-450's in the different liver samples.[125] Moreover, 7,8-benzoflavone did not affect the hydroxylation of coumarin or hexobarbital, thereby indicating the existence of different monooxygenases for the metabolism of these substrates. Multiple forms of cytochrome P-450 have been shown in the livers of rats, rabbits, and mice, but not thus far in humans.[124] More importantly, however, MCA is a potent inducer of BaP hydroxylase activity in rats but does not stimulate antipyrine hydroxylase, clearly suggesting that metabolism of POM in rodents may be regulated by different enzyme systems than in humans.[124]

In contrast to the apparent multiplicity of cytochrome P-450-dependent enzyme systems for the oxidative metabolism of POM in man, a single epoxide hydrase with broad substrate specificity may be present in human liver.[122,126] Because the hydration of arene oxides may lead to the formation of dihydrodiol carcinogen precursors, the capacity of different humans to metabolize epoxides may affect cancer susceptibility. It is not known, however, if enhanced dihydrodiol formation would increase cancer risk or decrease cancer risk.

Thomas and Slaga[127] did not obtain a correlation of AHH induction with skin-tumor-inducing ability in mice for a series of unsubstituted hydrocarbons. Nevertheless, the highest AHH enzyme activity was found in the epidermal layer of the skin, which is the major point of contact with many environmental chemicals. These results may be interpreted to indicate that a chemical carcinogen may not necessarily induce its own bioactivation, but instead can be transformed into a reactive intermediate by virtue of increased AHH activity stimulated by other noncarcinogenic compounds.

Due consideration must also be given to the fact that, in addition to the initiation of resting cells by a chemical carcinogen, a promotion phase involving cell proliferation is also involved in skin carcinogenesis.[128] Therefore, although certain aromatic hydrocarbons are effective enzyme inducers, their bioactivated metabolites may function only as an initiator having no promoting ability. A potent complete carcinogen, however, will be transformed not only into a powerful tumor initiator but will also be able to interact with cellular membranes, alter genetic expression, and ultimately cause irreversible cell proliferation. These observations raise certain doubts concerning the validity and/or reliability of equating enzyme inducibility with carcinogenic potential for chemical agents. Further reinforcement of this opinion has been provided by Shulte-Hermann[129] who showed that cell proliferation is not a direct result of enzyme induction, even though both processes are normally coupled.

The further possibility that the genetics of AHH inducibility is organ-dependent as well as strain-dependent in animals has important implications for evaluating susceptibility to POM-induced cancers.[130] Most significant is the demonstration that pulmonary AHH may be inducible in all strains of mice, regardless of the inducibility of hepatic AHH. Since the respiratory epithelium represents a primary portal of entry for POM, AHH activity which is induced in this tissue may bear importantly on susceptibility to malignancy.

Enzyme induction by POM is not limited to AHH. Owens[131] recently demonstrated that MCA can induce hepatic UDP-glucuronosyltransferase activity in certain inbred strains of mice. This enzyme catalyzes the conjugation and excretion of POM substrates after they have first been oxygenated by AHH. The induction of this transferase activity and that of AHH was apparently regulated by a single genetic locus. However, transferase inducibility does not depend on AHH levels, but rather is stoichiometrically related to the concentration of a specific and common cytosolic receptor regulating both enzyme induction processes. Owens further demonstrated that AHH activity can be fully induced in certain mouse strains (e.g., by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) without greatly enhancing the transferase activity. Earlier studies had established that chrysene and chlorpromazine were potent inducers of AHH activity while having little effect on transferase activity.[132] Subsequent exposure to carcinogenic POM (i.e., MCA) could lead to maximal oxidative metabolism but little transferase-catalyzed removal of metabolites by glucuronic acid conjugation. This situation would be exacerbated by the fact that metabolites of MCA are incapable of further inducing the transferase activity. This effect may have considerable toxicologic significance in that highly reactive epoxides of POM formed by the action of AHH under these circumstances may not be adequately removed by glucuronidation. Thus, one must consider the total exposure to all environmental agents and their possible effect on critical enzymatic processes before attempting to assess the toxicologic impact of exposure to a specific POM.

6.2.6 Intracellular Binding of POM

Investigators have known for a number of years that, when applied to the skin of animals or added to cells in culture, carcinogenic POM's become covalently bound to tissue constituents.[133] The molecular basis for carcinogenic and cytotoxic consequences of exposure to POM was attributed to the binding of the compounds to nuclear DNA,[134,135] although binding occurs to RNA and protein as well.[136,137] In the regenerating rat liver, DMBA produced an inhibition of DNA synthesis accompanied by a long-lasting (more than 4 weeks) DMBA-DNA binding.[135] In contrast, benz[a]anthracene, a weak carcinogen, inhibited DNA synthesis only slightly, and benz[a]anthracene binding to DNA was greatly diminished between 11 and 24 hours after administration. It was suggested that cells in the S phase of the cell cycle were primarily affected by the carcinogen. Similar effects on DNA synthesis were obtained using single applications of initiating doses of DMBA or DBahA to the skin of mice.[138] The weak tumor initiator, DBacA, had little effect on DNA synthesis. In mouse skin, it was also shown that DMBA preferentially binds to nonreplicating DNA.[139] Moreover, DNA-bound DMBA moieties could still function as templates for further DNA synthesis.[140] This finding suggested that newly synthesized abnormal daughter DNA may be genetically altered by an error-prone post-replicative repair process which leads to mutations and/or carcinogenesis. Such a hypothesis supports the somatic mutation theory of carcinogenesis.[141]

Binding of a carcinogen to DNA is believed to be a critical step in tumor initiation. The application of BaP to mouse epidermis resulting in the formation of BaP-DNA adducts has been used in examining dose-response relationships for BaP tumorigenesis. Albert and coworkers[142] found that the formation of BaP adducts was a linear function of dose between 4 and 100 nmoles/mouse. Since BaP carcinogenesis in mouse skin was a linear function between 16 and 500 nmoles/mouse, binding to DNA may allow for extrapolation of the dose-response curve down to levels which are unsuitable for carcinogenesis studies with limited numbers of animals. Furthermore, it allows one to evaluate the biological effects of environmentally realistic exposures to a particular POM. Lutz and coworkers[143] noted that over a dose range of 40 μg/kg to 4 mg/kg (approximately 50-5000 nmoles/animals, given i.p.) the binding of BaP to rat liver DNA was linear with doses up to about 1 mg/kg. A nonlinear dose-response relationship between 1 mg/kg and 2 mg/kg was evident, which could have been caused by an induction of microsomal enzyme activity. Above 2 mg/kg, microsomal enzyme activity was further enhanced, while DNA binding increased only slightly.

Because polycyclic aromatic hydrocarbons are not highly reactive chemically, it was believed that a reactive metabolite was responsible for the DNA-binding phenomenon. Gelboin[144] and Grover and Sims[145] independently confirmed this hypothesis by demonstrating that polycyclic hydrocarbons bind covalently to DNA in the presence of rat liver microsomes. The low level of binding for BaP to DNA (one molecule per 50,000 to 500,000 nucleotides) suggested the possibility of a unique binding site for BaP derivatives on the DNA.[144] Subsequent studies conducted *in vitro* with hamster tracheal epithelial cells incubated in the presence of 7,8-benzoflavone (an affector of microsomal drug-metabolizing enzymes) confirmed that BaP binding to DNA was dependent on an intact drug-metabolizing system.[146,147] In a related study, the binding of

BaP and MCA to rat liver DNA in vitro was enhanced in the presence of an epoxide hydrase inhibitor.[148] It is now known, however, that both aryl hydrocarbon hydroxylase and epoxide hydrase are required for the metabolic activation and subsequent binding of BaP to DNA.[149] These data implied that active epoxide intermediates are involved in the binding of polycyclic carcinogens to DNA, although the extent of binding need not correlate with carcinogenic potency. Others have argued, however, that a 6-oxybenzo[a]pyrene free radical may be involved in the binding of BaP to DNA and synthetic polynucleotides.[150-153] It was suggested that metabolic hydroxylation of BaP at position 6 leading to the formation of the 6-oxy BP radical may be only a minor route for binding to DNA.[154]

The role of K-region arene oxides as reactive intermediates in the metabolism of polycyclic hydrocarbons has been extensively reviewed,[43,58,155] although the structure of the bound material was not known at the time. Further confusion resulted from the demonstration that high levels of non-enzymatic binding of polycyclic hydrocarbons to DNA occurred with in vitro microsomal systems.[156] Nevertheless, extensive microsome-dependent binding of BaP to synthetic polynucleotide acceptors has been shown, which could be both inhibited or enhanced by conditions which affect microsomal enzyme activity.[156,157] Similar studies using DMBA-epoxides revealed that covalent complexes formed primarily with poly G, indicating that guanosine residues in nucleic acids may be preferred targets for reactive arene oxides.[158]

Systematic studies were undertaken to elucidate the relative role of K-region epoxides in binding reactions with nucleic acids. Blobstein and co-workers[159] examined the in vitro binding of both K- and non K-region arene oxides to poly G by measuring changes in the absorption and fluorescence spectra of the modified nucleic acid. Reactivity with nucleic acids was found to vary considerably, depending upon the structure of the parent hydrocarbon and position of the epoxide moiety. Significant binding to poly G occurred with K-region arene oxides of DMBA, BaP, and MCA (all strong carcinogens) and benz[a]anthracene (a weak carcinogen). K-region arene oxides of the noncarcinogens pyrene and phenanthrene produced no changes in the absorption spectra for poly G. However, the non-K-region BaP 9,10-, BaP 7,8-, and phenanthrene 3,4-oxides also produced alterations in poly G as measured by changes in fluorescence spectra. Further investigations revealed that RNA adducts forms when cultured bronchial mucosa is exposed to BaP[160] or when BaP is painted on mouse skin[161] are produced when BaP 7,8-diol-9,10-epoxides react at the 2-amino group of guanosine. Whereas Weinstein and coworkers[160] observed the formation of an adduct only between RNA and (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, Moore and coworkers[162] found that (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene also reacted with RNA _in vivo_ by cis and trans addition. The 7β,8α-diol-9β,10β-epoxide arises mainly from the (+)-enantiomer of BaP 7,8-dihydrodiol while the (-)-enantiomer produces primarily the 7β,8α-diol-9α,10α-epoxide (see Section 6.2.2). This observation explains why both AHH and epoxide hydrase are required for BaP binding to DNA, since BaP 7,8-oxide must be reduced by epoxide hydrase to form BaP 7,8-dihydrodiol, which is further metabolized to the BaP 7,8-diol-9,10-epoxide by the microsomal monooxygenases. Thus, the K-region BaP 4,5-oxide does not appear to be responsible for most of the observed covalent binding of BaP to RNA. In addition, examination of DNA adducts to BaP formed in vitro with Syrian hamster embryo cells,[163,164,165] cultured human bronchi,[149] BHK 21/C13 cells in culture,[166] or mouse liver microsomes[167] corroborated the results obtained for RNA, and disputed the involvement of a BaP K-region oxide in the binding reaction. Further support for the importance of BaP 7,8-diol-9,10-epoxides in the binding of BaP to nucleic acids was provided by studies which demonstrated that BaP 7,8-dihydrodiol is highly carcinogenic, and both diastereomeric BaP 7,8-diol-9,10-epoxides are potent mutagens (see Sections 6.4 and 6.5). A diol-epoxide of DMBA was also shown to be involved in the binding reaction with DNA of mouse embryo cells.[108,168]

Not all investigators agree that polycyclic hydrocarbon binding to DNA represents the essential interaction leading to mutagenesis/carcinogenesis. Sarrif and coworkers[169] were able to purify a protein ("h-protein") from C3H mouse liver and skin which could covalently bind to metabolites of MCA. The protein was postulated as a primary target in hydrocarbon-induced carcinogenesis, playing a similar role to hormone receptors in transporting carcinogens to a target site(s) in the nucleus. Alternatively it was suggested that the protein-carcinogen conjugate may in itself act as an abnormal derepressor to give rise to tumor formation. In related studies, MCA and DBahA were found to bind with high affinity to a nuclear subfraction from cultured mouse embryo cells.[170] This subfraction contained 15 percent of the nuclear DNA and represented that fraction of the nuclear chromatin which is transcriptionally active. The specific macromolecule responsible for the binding could have been, in addition to DNA, either RNA or one of the chromatin proteins involved in gene regulation. In support of the possible involvement of polycyclic carcinogens with essential chromatin components, it was noted that the weak carcinogen DBacA exhibited minimal binding to this same nuclear subfraction. Other investigators[171] incubated BaP with isolated calf thymus nuclei in the presence of NADPH and rat liver microsomes and demonstrated that an uneven distribution of carcinogen in chromatin occurs. The data suggested a localization of carcinogen in the outermost "spacer" regions of DNA in the nucleosome.

6.3 TOXICOLOGY

The potential for POM to induce malignant transformation dominates the consideration given to health hazards resulting from exposure. Although the emphasis on carcinogenicity is certainly justified when dealing with public health issues concerning POM, one must recognize that non-neoplastic lesions may also result from environmental and occupational contact. Such effects can be seen with subthreshold doses of carcinogenic POM and with those compounds which possess no tumorigenic activity.

As long ago as 1937, it was known that carcinogenic POM produced systemic toxicity as manifested by an inhibition of body growth in rats and mice.[172] Tissue damage resulting from the administration of various POM to experimental animals is often widespread and severe, although selective organ destruction may occur (e.g., adrenal necrosis, lymphoid tissue damage). Few investigators, however, have attempted to ascertain the molecular mechanism of POM-induced cytotoxicity. Nevertheless, current opinion favors the concept that normally proliferating tissues (intestinal epithelium, bone marrow, lymphoid organs, testis) are preferred targets for POM, and this susceptibility may be due to a specific attack on DNA of cells in the S phase of the mitotic cycle.[173] Additional factors which may have an important bearing on the adverse effects resulting from POM exposure are primary and secondary alterations in enzyme activity and immunologic competence. Moreover, these toxicant-induced changes may play an important role in the eventual induction of neoplasia.

6.3.1 Acute Exposures

Target organs for the toxic action of POM are diverse, due partly to extensive distribution in the body and also to the selective attack by these chemicals on proliferating cells. Damage to the hematopoietic and lymphoid systems in experimental animals is a particularly common observation. Yashuhira[174] described severe degeneration of the thymus and marked reduction in weight of the spleen and mesenteric lymph nodes of CF1 Swiss and C57BL mice given a single intraperitoneal injection of MCA (0.3-1.0 mg) between 12 hours and 9 days after birth. Degeneration of young cells in the bone marrow and retardation of thyroid gland development were also noted. Newborn mice were highly susceptible to the toxic effects of MCA, with many animals dying from acute or chronic wasting disease following treatment. Among surviving CF1 mice, numerous thymomas eventually developed; none were evident, however, in C57BL mice despite serious thymic damage.

DMBA is well-known for its effects on the bone marrow and lymphoid tissues. With single feedings (112 or 133 mg/kg B.W.) to female Sprague-Dawley rats, age 50 days, DMBA induced pancytopenia by causing a severe depression of hematopoietic and lymphoid precursors.[175] Maturation arrest occurred at the proerythroblast levels; no injury to the stem cells or the formed elements in the peripheral blood was evident. The fact that only the more rapidly proliferating hematopoietic elements were vulnerable to attack by DMBA led the authors to suggest that inhibition of DNA replication may be involved in the toxicologic response.

Philips and coworkers[173] provided strong support for the argument that DMBA-induced cytotoxicity is mediated via an interaction with DNA. Female

Sprague-Dawley rats receiving 300 mg/kg B.W. DMBA orally and male rats receiving an intravenous injection of 50 mg/kg B.W. DMBA displayed injury to the intestinal epithelium, extreme atrophy of the hematopoietic elements, shrinkage of lymphoid organs, agranulocytosis, lymphopenia, and progressing anemia. Mortality among rats receiving DMBA by gastric intubation (females) was about 65 percent; the group treated by intravenous injection showed about 25 percent mortality. In rats given 50 mg/kg B.W. DMBA intravenously, incorporation of ^{14}C-labeled thymidine into DNA of small and large intestine, spleen, bone marrow, cervical lymph nodes, thymus, and testis was significantly inhibited. This inhibition was as high as 90 percent in several organs at 6 hours, and indicated a strong inhibition of DNA synthesis. Consequently, the authors postulated that DNA in S phase cells is particularly susceptible to DMBA attack. This contention probably holds true for other carcinogenic POM as well.

Another lesion which is characteristic of that produced by X-rays is the severe testicular damage induced by DMBA in rats.[176] Single intravenous injections of DMBA (0.5 to 2.0 mg) given to adolescent (25 days of age) rats caused transient degenerative changes in the testis which were most evident 38 to 40 days after treatment. Essentially the same effects were produced in adult rats, age 60 days, given DMBA orally (20 mg) and intravenously (5 mg). Lesions of the testes were highly specific and involved destruction of spermatogonia and resting spermatocytes, both of which are the only testicular cells actively synthesizing DNA. Neither the remaining germinal cells nor the interstitial cells were damaged by DMBA. Surprisingly, no testicular damage was produced by single feedings of BaP (100 mg), MCA (105 mg), or 2-acetoaminophenanthrene (40 mg).

For many years it has been known that the application of carcinogenic polycyclic hydrocarbons to mouse skin leads to the destruction of sebaceous glands, hyperplasia, hyperkeratosis, and even ulceration.[177] Sebaceous glands are the skin structures most sensitive to polycyclic hydrocarbons, and assay methods for detection of carcinogens have been based on this effect. Although a good correlation can be obtained between carcinogenic activity and sebaceous gland suppression for many POM's (e.g., MCA, DMBA, BaP, DBahA, benz[a]anthracene), such an effect is neither necessary nor sufficient for carcinogenesis. However, workers exposed to POM-containing materials such as coal tar, mineral oil, and petroleum waxes are known to show chronic dermatitis, hyperkeratoses, etc.,[178,179] and the possible significance of these skin disorders to human cancer is not known.

In female animals, ovotoxicity has been reported to result from administration of POM. DMBA was shown to cause the destruction of small oocytes and reduced the numbers of growing and large oocytes after oral administration to mice.[180] More recently it was reported that destruction of primordial oocytes in mice by injection of MCA was correlated with the genetic capability for POM-induced increases in ovarian aryl hydrocarbon hydroxylase activity.[181] Thus, it is apparent that ovarian metabolism of POM and ovotoxicity are linked and are under genetic control.

A toxic reaction which is apparently unique to DMBA is the selective destruction of the adrenal cortex and induction of adrenal apoplexy in rats.[182] Adrenal apoplexy, increased adrenal gland weight, and increased month of treatment and semithin sections of the bronchi were examined microscopically. In the first animals sacrificed, minimal focal cell proliferation in the area of the basement membrane was evident in the bronchial epithelium. By 7 weeks, cytoplasmic vacuolization of both goblet and ciliated cells had occurred. Epithelial and basal cell proliferation continued for several weeks and led to the formation of three- to four-layered hyperplastic regions by the 11th week. Epithelial cells began to penetrate through the basement membrane by the 12th week, and within 2 more weeks the bronchial epithelium began to continuously grow into the surrounding lung tissues. Microscopic bronchogenic adenomata had developed by the 20th week. These tumors consisted primarily of ciliated cells and goblet cells, with only a few basal cells present. The apparently small amount of basal cell proliferation may have been the reason why squamous metaplasia was not observed by the time the experiment had ended after 21 weeks. Squamous metaplasia and keratinization were found in the trachea, but not in the bronchi, after 21 weeks of treatment. Although these investigators found no increase in the number of alveolar macrophages, others have reported numerous alveolar macrophage responses in BaP-treated hamsters as well as focal areas of accumulated macrophages containing a yellow pigment.[187-188]

Epithelial proliferation and cell hyperplasia in the absence of necrosis and/or marked inflammation is a common observation in the tracheobronchial mucosa of animals directly exposed to carcinogenic POM. This phenomenon was shown with repeated exposures of DB[c,g]C, DMBA, BaP, and dibenzo[a,i]pyrene in hamsters.[186,188-190] adrenal hemoglobin content were induced in female Sprague-Dawley rats by a single intragastric dose of 30 mg DMBA. The same amount of adrenal damage could be produced by a 5 mg dose of the principal DMBA oxidative metabolite, 7-hydroxymethyl-12-methylbenz[a]anthracene. Other DMBA metabolites produced no adrenal damage, thus indicating that a specific reactive intermediate may be responsible for this phenomenon.

6.3.2 Subchronic and Chronic Exposures

Repeated injections of benz[a]anthracene derivatives to mice and rats have produced prominent changes in the lymphoid tissue. Early investigators administered DBahA, benz[a]anthracene, and anthracene to mice in weekly subcutaneous injections for 40 weeks.[183] Each animal received a total dose of 10 mg, and 19 to 38 mice were treated with each of the three hydrocarbons. Analysis of lymph glands removed at weekly intervals showed an increase of reticulum (stem) cells and an accumulation of iron in all treatment groups. Lymphoid cells were reduced and lymph sinuses dilated in all groups, although these effects were more common in mice receiving DBahA. The weights of the spleens in mice treated with DBahA were significantly reduced in comparison to controls and those animals receiving benz[ah]anthracene or anthracene.

A more detailed study of the subchronic effects of DBahA on lymph nodes of male rats was reported in 1944.[184] Subcutaneous injections given five times weekly for several weeks caused normal lymph nodes to undergo hemolymphatic changes. These changes are characterized by the presence of extravascular red blood cells in the lymph spaces and the presence of large pigmented cells. These changes were not observed by Hoch-Ligeti[183] in mice, but could be produced in rats by BaP and MCA in addition to DBahA. The noncarcinogen anthracene, on the other hand, did not produce as dramatic a change in the lymph nodes of rats.

Since a likely route of environmental exposure to POM is by inhalation, there is considerable interest in its effect on the respiratory epithelium. In light of the concern over POM-induced neoplasms of the respiratory tract (see Section 6.7), an understanding of early pathological alterations and pre-neoplastic lesions in this tissue has particular significance.

Repeated intratracheal applications of DMBA or BaP (100 μg in gelatin or mineral oil) to male and female hamsters over periods from 4 to 16 months resulted in significant mortality (60 percent) before the appearance of lung tumors.[185] All groups of treated hamsters commonly displayed acute pneumonia and chronic pneumonitis. The latter condition was associated with a proliferation of large squamoid alveolar cells, which occasionally formed tissue masses filling one or more air spaces. As this proliferating alveolar epithelium began to destroy the adjacent reticulin framework, the lesions were considered to be malignant. Damage to the trachea occurred in practically all animals and consisted of mucosal and submucosal chronic exudative inflammation, ulceration, hyperplasia of reserve cells, squamous metaplasia, and squamous cell carcinoma (in the DMBA group only).

In a subsequent study conducted by Reznik-Schüller and Mohr,[186] BaP-induced damage to the bronchial epithelium of Syrian golden hamsters was examined in detail using semithin (1 μm) tissue sections. Animals were treated intratracheally with 0.63 mg BaP (total dose) dispersed in a solution of saline, dodecylsulfate, Tris-HCl, and EDTA once weekly for life. Animals were serially sacrificed at weekly intervals following the first month of treatment and semithin sections of the bronchi were examined microscopically. In the first animals sacrificed, minimal focal cell proliferation in the area of the basement membrane was evident in the bronchial epithelium. By 7 weeks, cytoplasmic vacuolization of both goblet and ciliated cells had occurred. Epithelial and basal cell proliferation continued for several weeks and led to the formation of three- to four-layered hyperplastic regions by the 11th week. Epithelial cells began to penetrate through the basement membrane by the 12th week, and within 2 more weeks the bronchial epithelium began to continuously grow into the surrounding lung tissues. Microscopic bronchogenic adenomata had developed by the 20th week. These tumors consisted primarily of ciliated cells and goblet cells, with only a few basal cells present. The apparently small amount of basal cell proliferation may have been the reason why squamous metaplasia was not observed by the time the experiment had ended after 21 weeks. Squamous metaplasia and keratinization were found in the trachea, but not in the bronchi, after 21 weeks of treatment. Although these investigators found no increase in the number of alveolar macrophages, others have reported numerous alveolar macrophage responses in BaP-treated hamsters as well as focal areas of accumulated macrophages containing a yellow pigment.[187-188]

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6.3.3 Effects on the Immune Systems

Numerous investigators have demonstrated that carcinogenic POM can produce an immunosuppressive effect. This action was first observed by Malmgren and coworkers[191] in 1952 using high doses of MCA and DB[a,h]A in mice. Subsequent studies established that single carcinogenic doses of MCA, DMBA, and BaP caused a prolonged depression of the immune response to sheep red blood cells.[192,193] Noncarcinogenic hydrocarbons such as benzo[e]pyrene and anthracene reportedly had no immunosuppressive activity. In a recent review on immunosuppression and chemical carcinogenesis, substantial evidence was presented to indicate that the degree of immunosuppression was correlated with carcinogenic potency for POM.[194] Both cell-mediated and humoral immune reactions are affected by POM.

The cellular mechanism of immunosuppression by POM has been investigated by several groups of researchers in recent years. Rowland and Hurd[195] reported that spleens from DMBA-treated (1 mg in 0.2 ml corn oil by single intramuscular injection) mice are deficient in bone marrow-derived lymphocytes but not in thymus-derived lymphocytes. Since the marrow-derived cells are short-lived and quickly replaced by the rapidly dividing marrow-derived lymphocyte population, it was suggested that DMBA preferentially attacked this population. Because immune capacity was determined only 7 days after DMBA treatment, an effect on the long-lived thymus-derived cells could probably not have been seen. This explanation was supported by data from Yamashita and coworkers[196] who analyzed the effects of DMBA on marrow- and thymus-derived lymphocytes of mice up to six weeks after exposure. In these studies, a bimodal immunosuppressive effect was obtained--a short-acting depression of marrow-derived cells and a long-lasting depression of thymus-derived cells could be seen.

In contrast to the results presented above, long-term (6 to 9 months) exposure of guinea pigs to MCA (4 mg every 4 weeks) resulted in no sustained suppression of cell-mediated or humoral immunity.[197] These investigators suggested that immunosuppression resulting from exposure to POM is a function of its general toxic effect, but does not persist long enough to be involved in neoplastic induction.

The importance of an intact immune system in resisting the effects of chemical carcinogens is debatable. Immunosuppression induced by thymectomy or treatment with antilymphocyte serum has a more dramatic effect on carcinogenesis induced by viruses than that induced by chemicals.[194] Moreover, a dissociation between the carcinogenic and immunosuppressive effects of MCA was shown at low doses in C3H mice.[198] That is, single doses of MCA less than 0.1 mg induced subcutaneous sarcomas in many treated animals in the absence of serum antibody responses to sheep red blood cells. In addition, one-time administration of MCA intragastrically (0.2 to 2.0 mg) to C3H mice was carcinogenic but had no effect on immune responses.

6.4 CARCINOGENESIS

Numerous polycyclic aromatic compounds are distinctive in their ability to produce tumors in skin and most epithelial tissues of practically all species tested. Malignancies are often induced by acute exposures to microgram quantities of POM. Latency periods can be short (4-8 weeks) and the tumors produced may resemble human carcinomas. Carcinogenesis studies involving POM have historically been directed at studies primarily involving effects on the skin or lungs. In addition, subcutaneous or intramuscular injections are frequently employed to produce sarcomas at the injection site. Ingestion has not been a preferred route of administration for the bioassay of POM, despite the fact that it is a major route of human exposure (see Section 5.5).

Concern over potential human cancer risk posed by POM present in the atmosphere stems from studies demonstrating that crude extracts of airborne particulate matter and automotive exhaust were carcinogenic to animals.[199-204] Fractions soluble in benzene or benzene-methanol produced tumors in mice by skin painting or subcutaneous injection, and in hamsters by intratracheal instillation. Both the aromatic and oxygenated neutral subfractions of the airborne particulate were active as complete carcinogens, and indicated the presence of numerous carcinogenic materials, including non-POM. The further demonstration that the basic fraction of organic pollutants was carcinogenic to newborn mice indicated the presence of aza-heterocyclic hydrocarbons. Since the carcinogenicity of the total organic particulates and aromatic neutral subfractions could only partly be explained by the presence of BaP, its usefulness as a measure of carcinogenic risk may, therefore, be limited.

From investigations in which polycyclic carcinogens were painted on the skin of mice has emerged the two-stage theory of skin carcinogenesis.[205,206] The first stage, initiation, results from the ability of a carcinogen to effect a permanent change within a cell or cell population following a single application. The measure of carcinogenic potency is often regarded as the capacity for tumor initiation. However, some weak or non-carcinogens can be active as tumor initiators (e.g., dibenz[a,c]anthracene, 1-methylchrysene, benz[a]anthracene). The second stage, promotion, is a prolonged process which does not necessarily require the presence of a carcinogen, but nevertheless a chemical stimulus must be supplied (e.g., by the phorbol esters contained in croton oil). A complete carcinogen is one which, if applied in sufficient quantity, can supply both initiating and promoting stimuli (e.g., DMBA, BaP). The formation of skin tumors by polycyclic hydrocarbons may also be influenced by inhibitors and accelerators (cocarcinogens), thus complicating the interpretation of experimental data.

The carcinogenic effects of POM when applied to the skin of animals have been known for decades. In 1939, Iball[207] collected the results of a series of experiments to arrive at a method for comparing the carcinogenic potencies of various polycyclic aromatic chemicals. His results, presented in Table 6-15, express tumorigenic potency in mouse skin as the ratio of percent tumor incidence to the average latency period. This expression, commonly referred to as the Iball index, is still used as a means of comparing the relative activity of carcinogens. An important data compilation

Table 6-15. CARCINOGENIC COMPOUNDS IN DESCENDING ORDER OF POTENCY.[207] [a]

Compound	Number of mice alive when first tumor appears	Number of tumors	Percentage of tumors (A)	Papilloma	Epithelioma	Average latent period (B)	Index (A/B x 100)
1. 7,12-Dimethylbenz[a]anthracene	20	13	65	6	7	43	151
2. 3-Methylcholanthrene (a)	18	18	100	1	17	99	101
3. 3-Methylcholanthrene (b)	8	5	62.5	0	5	151	41
4. 3-Methylcholanthrene (a and b added together)	26	23	88.5	1	22	109	80
5. Benzo[a]pyrene (from pitch)	10	10	100	2	8	127	79
6. Benzo[a]pyrene (synthetic)	9	7	78	2	5	109	72
7. Benzo[a]pyrene (5 and 6 added together)	19	17	89.5	4	13	119	75
8. Cholanthrene	49	28	57	5	23	112	51
9. 5,6-cycloPenteno-benz[a]anthracene	14	13	93	1	12	194	48
10. 2-Methyl-benzo[c]phenanthrene	16	12	75	5	7	155	48
11. 10-Methyl-benz[a]anthracene	18	12	66.5	2	10	147	45
12. 5,6-Dimethyl-benz[a]anthracene	19	16	84	0	16	220	38
13. 6-isoPropyl-benz[a]anthracene	15	11	73.5	1	10	204	36
14. Dibenzo[c,g]carbazole	19	9	47.5	4	5	143	33
15. Dibenzo[a,h]pyrene	17	10	59	0	10	205	29
16. 5-Methyl-benz[a]anthracene	8	7	87.5	2	5	317	26
17. 5-Ethyl-benz[a]anthracene	9	7	77.5	2	5	285	27
18. Dibenz[a,h]anthracene	65	41	63	8	33	239	26
19. Benzo[c]phenanthrene	18	12	67	5	7	387	17
20. Dibenzo[a,g]carbazole	9	4	44.5	1	3	263	17
21. 5-n-Propyl-benz[a]anthracene	20	6	30	3	3	192	16
22. Dibenz[c,h]acridine	28	11	39.3	2	9	357	11
23. 3-Methyl-dibenz[a,h]anthracene	25	7	28	1	6	325	9
24. Dibenz[a,h]acridine	25	6	24	2	4	350	7
Totals		305		60	245		

[a] compounds applied by skin painting

on agents tested for carcinogenicity has more recently been published by the U.S. Public Health Service (Publication No. 149) which lists the results of tests on hundreds of chemicals in numerous species including rodent, avian, and amphibian.

Experimental models for respiratory carcinogenesis have major limitations in that the delivery of carcinogens to the tracheobronchial tree in measured amounts and their adequate retention at the target tissue are poorly controlled. Therefore, the conduct of dose-response studies for lung tumor induction has been seriously hampered. Moreover, the possible relevance of the two-stage theory of carcinogenesis to lung cancer has not been clearly established. Much of the bioassay data on POM-induced lung cancer has been derived from animal model systems employing various modes of administration (inhalation, intratracheal instillation, intravenous injection), and the use of carrier particles (e.g., ferric oxide) for the delivery of the carcinogen to the bronchial epithelium. Thus, the results obtained from these studies cannot always be directly compared. The most commonly employed method for the study of POM-induced lung cancer involves intratracheal instillation of test material in the Syrian golden hamster.

6.4.1 Structure-activity relationships

Following the identification of the first carcinogenic hydrocarbon from soot, BaP, an intensive effort was mounted to isolate the various active components of carcinogenic tars.[208] From the earliest studies conducted, the realization emerged that carcinogenic POM are structurally derived from the simple angular phenanthrene nucleus.[209] However, unsubstituted POM with less than four condensed rings that have been tested have not shown tumorigenic activity. Furthermore, of the six possible arrangements with four benzene rings, several of these compounds are active: benzo[c]phenanthrene, benz[a]anthracene and chrysene. The unsubstituted penta- and hexacyclic aromatic hydrocarbons are clearly the most potent of the series. These include BaP, DBahA, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, benzo[b]fluoranthene, and benzo[j]fluoranthene. Somewhat less potent as carcinogens are the dibenzanthracenes and dibenzophenanthrenes. Only a few heptacyclic hydrocarbons show carcinogenic activity. These include phenanthro-[2',3':3,4']pyrene peropyrene and dibenzo[h,rst]pentaphene. Beyond seven unsubstituted aromatic rings, there are very few known carcinogenic hydrocarbons. However, many physical-chemical and enzymatic parameters must be dealt with when speaking of carcinogenic POM. Factors such as solubility with increasing molecular size and intracellular localization to achieve metabolic activation are likely to be important determinants of the true carcinogenicity of a particular POM.

Among the unsubstituted polycyclic hydrocarbons containing a nonaromatic ring, a number of active carcinogens are known. The most prominent examples of this type of compound are cholanthrene, 11,12-ace-benz[a]anthracene, 8,9-cyclopentanobenz[a]anthracene, 6,7-ace-benz[a]anthracene, acenaphthanthracene, 1,2,5,6-tetrahydrobenzo[j]cyclopent[f,g]aceanthrylene, and "angular" steranthrene. All of these compounds retain an intact conjugated phananthrene segment.

The addition of alkyl substituents in certain positions in the ring system of a fully aromatic hydrocarbon will often confer carcinogenic activity or dramatically enhance existing carcinogenic potency. In this regard, Arcos and Argus[209] noted that monomethyl substitution of benz[a]anthracene can lead to strong carcinogenicity in mice, with potency depending on the position of substitution in the decreasing order, 7>6>8=12>9. A further enhancement of carcinogenic activity is produced by appropriate dimethyl substitution of benz[a]anthracene. Active compounds are produced by 6,8-dimethyl-, 8,9-dimethyl-, 8,12-dimethyl-, 7,8-dimethyl-, and 7,12-dimethyl-substitution. The latter compound is among the most potent POM carcinogens known, although it has not been shown as a product of fossil fuel pyrolysis. Methyl substitution in the angular ring of benz[a]anthracene, however, tends to deactivate the molecule, although 4,5-dimethylbenz[a]anthracene may be an exception. Carcinogenic trimethyl- and tetramethylbenz[a]anthracenes are known, and their relative potencies are comparable to the parent 7,12-DMBA. In general, free radical synthesis of polycyclic hydrocarbons by pyrolysis does not favor alkyl side chain formation.

Alkyl substitution of partially aromatic condensed ring systems may also add considerable carcinogenic activity. The best example of this type of activation is MCA, a highly potent carcinogen.[209]

With alkyl substituents longer than methyl, carcinogenicity tends to decrease, possibly due to a decrease in transport through cell membranes. However, different positions in the benz[a]anthracene molecule will vary with respect to the effect of n-alkyl substitution on carcinogenicity. Benz[a]anthracene is especially sensitive to decreased carcinogenicity caused by the addition of bulky substituents at the 7-position, and is indicative of a once widely-held view for most polycyclics that high reactivity of the meso-phenanthrenic region (now called the "K-region") was a critical determinant for carcinogenicity.[209] Current studies show that the K-region is not involved in critical binding to DNA (see Section 6.2.6). The substitution of highly polar groups (e.g., -OH, -COOH) in the 7-position of benz[a]anthracene abolishes tumorigenic activity whereas a wide variety of less-polar substituents can enhance activity in position 7 (e.g., $-CH_2OH$, $-CH_2CH_2OH$, $-CH_2COONa$, $-CH_2COOCH_3$, $-CH_2OOCCH_3$, -CN, $-CH_2CN$, -CHO, $-NH_2$, -SH, $-COCCl_3$, $-OCH_3$).

Recent studies have indicated that methylation of the angular "bay region" (see Section 6.4.2) benzene ring not only in benz[a]anthracene but also in other four, five, and six-ring aromatic hydrocarbons leads to a significant decrease, or even to elimination, of the carcinogenic activity of the molecule. Methylation in other positions does not diminish, but frequently increases, carcinogenicity. For example, 7- and 8-methyl-BaP are inactive, whereas 2-,3-,4-,5-,6-,11-, and 12-methyl-BaP are strong carcinogens.

Partial hydrogenation of the polycyclic aromatic skeleton can generally be expected to decrease carcinogenic potency. This was shown with various hydrogenated derivatives of BaP, benz[a]anthracene, and MCA. On the other hand, the carcinogenicity of DBahA, dibenzo[a,i]pyrene, and dibenzo[a,h]pyrene is not significantly altered by meso-hydrogenation. This may be due to the fact that extensive resonance capability is preserved. Moreover, 5,6-dihydro-DBahA actually displayed a fourfold increase in carcinogenicity in comparison to the parent hydrocarbon,[209] possibly due to the hydrophilicity and ease of intracellular transport of its dihydrodiol derivative.

Structure-activity relationships for nitrogen-containing heterocycles have not been as thoroughly investigated as for polycyclic hydrocarbons.[209] Nevertheless, several generalizations can be derived from available data. Arcos and Argus[209] observed that the benz[c]acridine nucleus is more likely to yield carcinogenic derivatives than benz[a]acridine. In addition, they noted that the general rule of increased activity resulting from methyl substitution on one of the meso-anthracenic carbons of benz[a]anthracene also applies to this series (i.e., position 10 in benz[c]acridine or position 9 for benz[a]acridine). Lengthening of the alkyl substituent or addition of a second methyl group to benz[c]acridine will not increase activity and may, in fact, abolish it. Dimethyl substitution of benz[a]acridine, on the other hand, appears to enhance carcinogenicity.

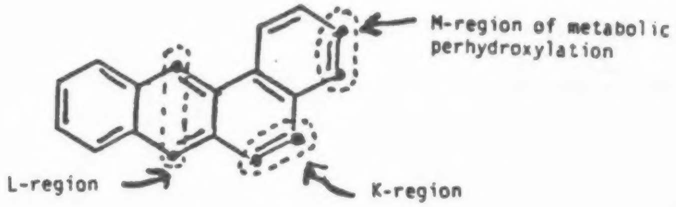
In the dibenzacridine series, carcinogenicity appears to decrease in the order dibenz[c,h]acridine > dibenz[a,h]acridine > dibenz[a,j]acridine, with all compounds being less active than the monomethyl- and dimethylbenz[a]-acridines. Addition of meso alkyl substituents will generally enhance carcinogenicity, but hydrogenation of the aromatic skeleton or addition of further benzene rings will substantially reduce or abolish activity.

Other POM carcinogens containing one or more nitrogen heteroatoms belong to the benzocarbazole series. Most prominent among these is dibenzo-[c,g]carbazole. However, dibenzo[a,g]carbazole also shows some carcinogenic activity. The activity of dibenzo[a,g]carbazole, however, is considerably less than for the isosteric benzopyridocarbazoles. Furthermore, although dibenzo[a,i]carbazole shows only very weak activity, introduction of a second nitrogen atom to form 7,8-benzo-pyrido[2',3':1,3]carbazole produces a dramatic potentiation of sarcomatogenic activity. While the acridines, carbazoles, and other nitrogen heterocycles have not been studied in as great detail as polycyclic hydrocarbons, their importance as potentially hazardous environmental pollutants is likely to increase in the future.

Greater emphasis on alternatives to solid fuel combustion (e.g., coal liquefaction) is expected to result in greater emissions of these nitrogen-containing compounds.

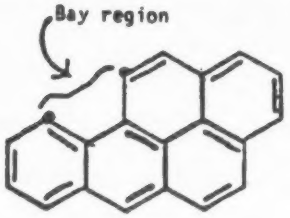
6.4.2 Chemical Reactivity and Carcinogenicity

For many years, investigators have sought a common molecular feature among POM carcinogens which would serve to explain their biological activity. The "electronic theory of carcinogenesis" has relied upon an analysis of the influence of electron density at specific molecular regions to explain unique reactivity with cellular constituents. A basic assumption arising from the work of the Pullmans[30] and others was that a meso-phenanthrenic region ("K-region") of high π-electron density and with a propensity for addition reactions was a critical structural feature for polycyclic carcinogens. In expanding this hypothesis, further biological significance was attributed to the concomitant presence of a rather unreactive meso-anthracenic region ("L-region") for high carcinogenicity. In addition, a region of comparatively low reactivity which characteristically undergoes metabolic perhydroxylation (corresponding to the 3,4-positions of benz[a]anthracene) has been designated the M-region. According to the theory, only binding of the K-region to critical cellular sites would cause tumor formation; protein binding at the L-region causes no tumorigenic effect, while inactivation is produced by metabolic perhydroxylation in the M-region. The three regions of reactivity are readily distinguished in the benz[a]anthracene skeleton:

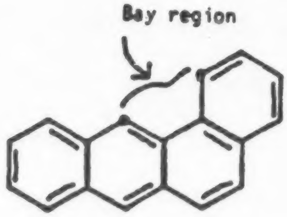


The electronic K-L theory of carcinogenic reactivity has encountered numerous inconsistencies, primarily because these relationships were derived from physical-chemical properties of the parent hydrocarbon and gave no consideration to the biological effects of activated metabolites.

Advances in recent years have focused attention on the potential reactivity of diol epoxide metabolites of POM, and their ease of conversion to triol carbonium ions. Under the assumption that diol epoxides, which are more readily converted to carbonium ions, will be better alkylating agents to produce carcinogenesis and mutagenesis, the "bay region" theory has been proposed.[31,64,211] Examples of a "bay region" in a polycyclic hydrocarbon are the regions between the 10 and 11 positions of BaP and the 1 and 12 positions of benz[a]anthracene:



Benzo[a]pyrene



Benz[a]anthracene

The theory predicts that diol epoxides in which the oxirane oxygen forms part of a "bay region" (e.g., BaP 7,8-diol-9,10-epoxide) will be more reactive and hence more carcinogenic than diol epoxides in which the oxirane oxygen is not situated in a "bay region." Experimentally, the "bay region" theory has been confirmed for benz[a]anthracene,[267,530,531,533,534,536,537] BaP,[66-68,72,73,538] chrysene,[535] and DBahA.[532,539] Moreover, quantum mechanical calculations were in accord with the concept that reactivity at the "bay region" is highest for all the diol epoxides derived from polycyclic hydrocarbons.

The bay region concept has received enough confirmation to lead to suggestions that an analysis of theoretical reactivity in this manner may be useful in screening POM as potential carcinogens.[212] Among several indices of theoretical reactivity examined, the presence of a bay region for a series of POM displayed a high degree of correlation with positive carcinogenic activity.[212]

6.4.3 Skin Carcinogenesis and Induction of Local Sarcomas

6.4.3.1 Benzo[a]pyrene--The ability of BaP and its various metabolic products to initiate skin tumors in the two-stage mouse carcinogenesis system has been extensively evaluated in recent years. Emphasis has been placed on the identification of a BaP derivative which acts as the principle ultimate carcinogen resulting from metabolic activation. Various BaP-oxides, -phenols, -dihydrodiols, and diol-epoxides were assayed for tumorigenicity and compared to the parent compound as a measure of bioactivation.[214-221,538]

The tumor response obtained when BaP alone is applied weekly to the skin of mice is shown in Figure 6-12.[142] It is apparent from the data presented that increasing weekly doses of BaP caused a shortening of the latency period for carcinoma formation. Furthermore, it was determined that the development of papillomas as a precursor lesion to carcinoma formation occurred only at higher BaP doses (e.g., 32 μg and 64 μg per week). At the lower dose levels (8 μg and 16 μg per week), carcinomas appeared *de novo* without precursor papilloma formation.

Among the arene oxides of BaP tested as complete carcinogens by repeated topical applications to mouse skin (0.4 μmole once every two weeks for 60 weeks), BaP 7,8-oxide was shown to be highly carcinogenic.[214] At lower doses (0.1 μmole), BaP 7,8-oxide was not as potent a carcinogen as BaP. The

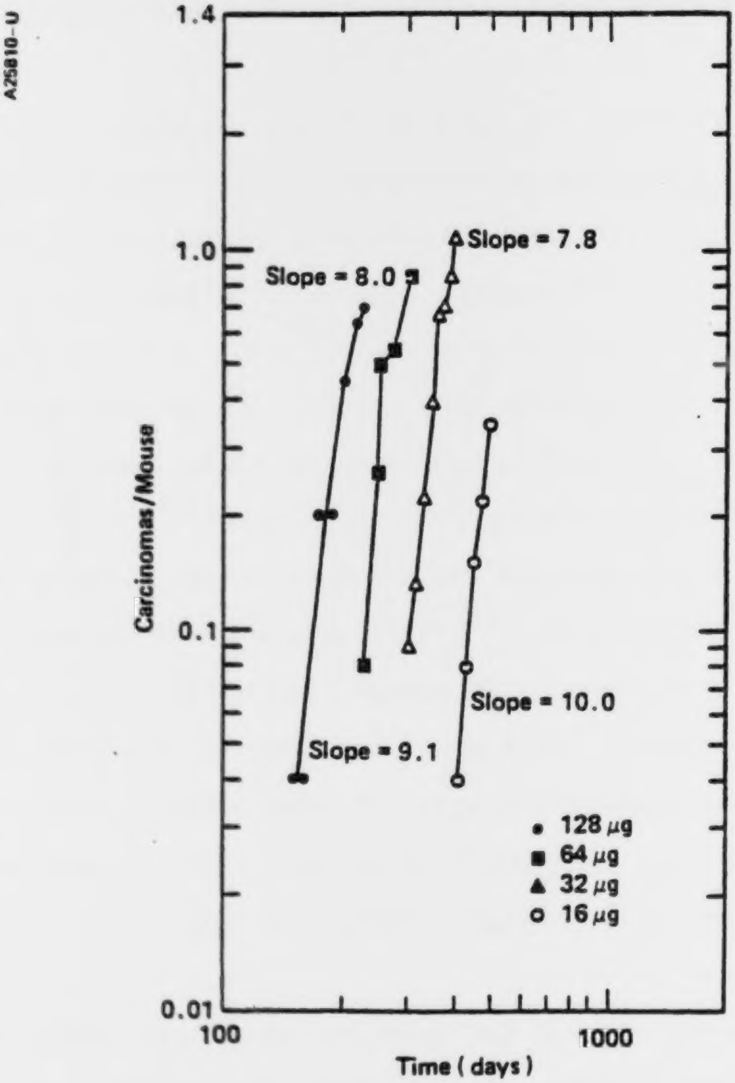


Figure 6-12. Log-log plot of the cumulative incidence of skin carcinomas per mouse versus time. BaP was applied topically every week at the indicated doses. Time was measured from the first BaP treatment.[142]

K-region BaP 4,5-oxide was only weakly carcinogenic at high doses, whereas BaP 9,10-oxide was inactive. Subsequent studies to determine if the phenolic non-enzymatic rearrangement products of BaP oxides were carcinogenic established that none of the seven phenols of BaP tested (4-,5-,6-,7-,8-,9-, and 10-hydroxybenzo[a]pyrene) were active.[219] Among the remaining five possible isomeric phenols of BaP, only 2-hydroxybenzo[a]pyrene was shown to be highly carcinogenic at the 0.4 μmole dose.[220] However, this compound has not been found as a metabolite of BaP in biological systems.

Following the demonstration of the carcinogenicity of BaP 7,8-oxide to mouse skin, Levin et al.[218] demonstrated that BaP 7,8-dihydrodiol, which can form in vivo by the action of epoxide hydrase on BaP 7,8-oxide, was even more potent than the parent epoxide. In fact, it was shown[217] that BaP 7,8-dihydrodiol was a slightly stronger skin carcinogen than BaP. In the same study it was established that the diastereomeric BaP 7,8-diol-9,10-epoxides were either inactive or weakly active as complete carcinogens on mouse skin.

A summary of the available information concerning carcinogenicity of BaP and its derivatives to mouse skin is presented in Table 6-16. It cannot be determined with certainty from these data, however, which products of BaP biotransformation may act as ultimate carcinogens. The apparent lack of carcinogenicity of the BaP 7,8-diol-9,10-epoxides to mouse skin, despite abundant evidence concerning their mutagenicity (see Section 6.5), binding to nucleic acids (see Section 6.2.6), and high carcinogenicity to newborn mice,[72,73] may be explained by poor penetration of the skin. Since it is a highly reactive electrophile, a BaP diol epoxide may readily alkylate nucleophilic sites of any kind and thus have a reduced probability of passing

Table 6-16. SKIN TUMORS IN MICE TREATED WITH BENZO[a]PYRENE AND DERIVATIVES

Treatment[a]	Total number of animals	Dose, µmoles	% of mice with tumors	Total number of skin tumors[b]	Reference
BaP	25	0.4	100	32	220
BaP	30	0.4	100	34	220
BaP	26	0.4	92	34	219
BaP	30	0.15	100	40	218
BaP	27	0.1	96	28	220
BaP	30	0.1	38	13	217
BaP	30	0.1	50	15	217
BaP	30	0.1	91	24	217
BaP	30	0.05	59	20	217
BaP	30	0.025	7	2	217
BaP	30	0.02	4	1	217
BaP	30	0.02	0	0	217
1-HOBaP	25	0.4	0	0	220
2-HOBaP	29	0.4	100	37	220
3-HOBaP	29	0.4	0	0	220
4-HOBaP[c]	26	0.4	0	0	219
5-HOBaP[c]	26	0.4	0	0	219
6-HOBaP[c]	28	0.4	0	0	219
7-HOBaP[c]	30	0.4	0	0	219
8-HOBaP[c]	27	0.4	0	0	219
9-HOBaP[c]	26	0.4	0	0	219
10-HOBaP[c]	28	0.4	0	0	219
11-HOBaP	28	0.4	14	4	220
12-HOBaP	23	0.4	0	0	220

[a] Female C57BL/6J mice were treated with BaP or BaP derivaties (0.02-0.4 µmole) once every two weeks for 60 weeks by topical application to the shaved skin of the back.

[b] Skin tumors consisted mostly of squamous cell carcinomas; other skin tumors were fibrosarcomas, papillomas, and keratocanthomas.

[c] Mice were treated once every two weeks for 56 weeks.

Table 6-16 (continued). SKIN TUMORS IN MICE TREATED WITH BENZO[a]PYRENE AND DERIVATIVES

Treatment[a]	Total number of animals	Dose, µmoles	% of mice with tumors	Total number of skin tumors[b]	Reference
BaP 4,5-oxide	30-39	0.4	4	1	214
BaP 4,5-oxide	30-39	0.1	6	2	214
BaP 7,8-oxide	30-39	0.4	94	37	214
BaP 7,8-oxide	30	0.3	53	16	218
BaP 7,8-oxide	30	0.15	18	5	218
BaP 7,8-oxide	30-39	0.1	9	3	214
BaP 9,10-oxide	30-39	0.4	0	0	214
BaP 11,12-oxide	28	0.4	0	0	220
BaP 11,12-oxide	27	0.1	0	0	220
BaP 7,8-dihydrodiol	30	0.3	100	42	218
BaP 7,8-dihydrodiol	30	0.15	100	40	218
BaP 7,8-dihydrodiol	30	0.1	92	28	214
BaP 7,8-dihydrodiol	30	0.05	76	24	214
BaP 7,8-dihydrodiol	30	0.025	7	2	214
(±)-7β,8α-Dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (diol epoxide 1)	30	0.4	0	0	214
diol epoxide 1	30	0.1	0	0	214
diol epoxide 1	30	0.02	0	0	214
(±)-7β,8α-Dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (diol epoxide 2)	30	0.4	13	3	214
diol epoxide 2	30	0.1	7	2	214
diol epoxide 2	30	0.02	0	0	214

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[a] Female C57BL/6J mice were treated with BaP or BaP derivatives (0.02-0.4 µmoles) once every two weeks for 60 weeks by topical application to the shaved skin of the back.

[b] Skin tumors consisted mostly of squamous cell carcinomas; other skin tumors were fibrosarcomas, papillomas, and keratocanthomas.

intact through the cytoplasm to reach critical cellular receptors, which most likely are in the nucleus.

The possibility that susceptibility to BaP-induced skin cancer may be related to age was examined by Peto and coworkers.[222] The incidence of skin cancer in mice resulting from regular BaP application (20 μg) starting at 10, 25, 40, or 55 weeks of age was related to duration of exposure and not to age at the start of treatment. This suggests that the induction of the carcinogen-activating enzyme system does not change with increasing age.

The activity of BaP and its derivatives as tumor initiators in the two-stage mouse skin carcinogenesis system has received considerable attention. The rationale for investigating the tumor-initiating capabilities of BaP derivatives stems from the knowledge that hydroxylated intermediates may be better substrates for activation to a proximate or ultimate carcinogen. Moreover, it would not be expected that all carcinogenic POM metabolites should be equally effective as tumor initiators, since different tissues or species may have greater or lesser capacity to activate a specific molecule.

Among the various metabolites of BaP investigated for tumor-initiating activity, only BaP 7,8-dihydrodiol is as potent a tumor initiator as BaP.[216] In addition, the tumorigenicity of BaP 7,8-dihydrodiol is highly stereospecific.[223] At equimolar doses, (-)-BaP 7,8-dihydrodiol was more active than BaP as a tumor initiator, whereas the (+)-enantiomer was considerably less active than BaP. Other effective tumor-initiating metabolites of BaP are BaP 7,8-oxide and BaP $7\beta,8\alpha$-diol-$9\beta,10\beta$-epoxide (diol epoxide 1).[215] However, both diol epoxide 1 and diol epoxide 2 (BaP $7\beta,8\alpha$-diol-$9\alpha,10\alpha$-epoxide) were severalfold more active than BaP in the induction of epidermal hyperplasia.[278] A summary of the skin tumor initiating activities of BaP and its metabolites is presented in Table 6-17.

Table 6-17. SUMMARY OF THE SKIN TUMOR INITIATING ACTIVITIES OF BENZO[a]PYRENE AND ITS METABOLITES

Initiator	Number of mice	Dose, nmoles	Weeks of promotion	% of mice with tumors	Papillomas/ mouse	Reference
Bap	30	200	23	94	4.8	216
BaP	30	200	30	92	5.3	215
BaP	30	100	21	77	2.6	223
BaP 4,5-epoxide	30	200	23	20	0.2	216
BaP 7,8-epoxide	29	200	23	81	1.9	216
BaP 9,10-epoxide	29	200	30	15	0.15	215
BaP 11,12-epoxide	30	200	30	38	0.45	215
BaP 7β,8α-diol-9α,10α-epoxide	29	200	30	69	1.5	215
BaP 7β,8α-diol-9β,10β-epoxide	28	200	30	7	0.07	215
BaP 7,8-dihydrodiol	29	200	30	86	5.0	215
(-)-BaP 7,8-dihydrodiol[b]	30	100	21	77	3.8	223
(+)-BaP 7,8-dihydrodiol[b]	30	100	21	23	0.43	223

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[a] Female CD-1 mice were treated with a single dose of initiator dissolved in acetone, acetone:NH_4OH (1000;1), or dimethyl sulfoxide:acetone (1:3) and followed one week later by twice-weekly applications of 10 μg of TPA.

[b] Promotion was by twice-weekly applications of 16 nmoles of TPA beginning 11 days after treatment with initiator.

Following the subcutaneous administration of BaP to rats and mice, sarcomas commonly develop at the site of injection since the carcinogen is introduced below the basal cell layer. A total dose of 6 to 7 μmoles (1.5 to 1.7 mg) of BaP is sufficient to produce sarcomas in nearly all treated animals (Table 6-18). Although a single dose of 0.31 mg or less of BaP produced no tumors in C3H mice, others reported that 0.0004, 0.004, and 0.04 mg produced sarcomas in one, five, and 23 mice (strain unknown), respectively, out of groups of 50.[208]

6.4.3.2 Benz[a]anthracene--The weakly carcinogenic activity of BA has been known for many years. Recent studies have attempted to apply the bay region theory (see Section 6.4.2) to explain the carcinogenicity of BA using mouse skin as the experimental bioassay system. As predicted by the bay region theory, the 3,4-dihydrodiol of BA and BA 3,4-diol-1,2-epoxide showed high tumorigenic activity on mouse skin, and act as proximate and ultimate carcinogens, respectively.[534,536,537] Further studies involving the production of pulmonary tumors in newborn mice with the diastereomeric BA 3,4-diol-1,2-epoxides and the (+) and (-) enantiomers of BA 3,4-dihydrodiol have confirmed the results shown in mouse skin.[531]

6.4.3.3 7,12-Dimethylbenz[a]anthracene--DMBA is a potent carcinogen used extensively in the laboratory. It is not found, however, in the environment. When applied topically, DMBA is both an effective tumor initiator and a complete carcinogen, depending upon the dose administered.[227-232] Numerous studies have indicated that a single dose of DMBA greater than 400 nmoles is sufficient to produce tumors in mouse skin in the absence of a promoting stimulus.[233] Attempts to produce skin tumors in CD-1 female mice with a

Table 6-18. INDUCTION OF SARCOMA BY BENZO[A]PYRENE

Species	Number and (sex)	Total dose, μmoles	Animals with sarcoma, %	Average latency, days	Reference
Rat (Sprague-Dawley)	13 (female)	6.0[a]	100	101 ± 2.7	224
Mouse	14 (male	7.1[b]	93	129	225
Mouse	16 (female)	7.1[b]	50	160	225
Mouse	9 (?)	15.9[c]	66.6	112	226
Mouse	10 (?)	5.0[c]	70	122	226
Mouse	12 (?)	0.5[c]	66.6	155	226
Mouse	15 (?)	.002[c]	0	N.A.[d]	226

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[a] Administered as 0.2 μmole dissolved in 0.1 ml sesame oil by subcutaneous injection on alternate days for 30 doses beginning at 30 days of age.

[b] Administered as three injections of 2.4 μmoles each, given at one month intervals.

[c] Administered as a single injection under the skin of the abdomen, dissolved in 0.5 ml of neutral olive oil.

[d] Not applicable.

single application of 200 nmoles of DMBA were unsuccessful after 35 weeks. DMBA is metabolized much faster than BaP and has a more complicated metabolite profile suggesting that more biochemical parameters may be modulating DMBA tumorigenesis.

Albert and coworkers[142] found that DMBA readily induces tumors on the skin of rats as well as mice. In general, the temporal and dose-response features of the action of DMBA on rat skin were very similar to that shown in mouse skin treated with BaP (Figure 6-13). The data suggested that a similar mechanism of action may be operating for these compounds in both species.

Not only are skin tumors induced by single topical applications of DMBA, they may also be formed after injection of the carcinogen. Bennington and coworkers[234] reported that six of 70 female Sprague-Dawley rats developed seven pilosebaceous tumors within one year after receiving 3 mg of DMBA by intravenous injection. Slaga and coworkers[233] reported that a single intraperitoneal injection (0.6-4.8 μmoles) of DMBA to mice, followed by repeated application of phorbol ester to the skin, caused the appearance of multiple papillomas.

When applied to the skin of guinea pigs, high doses of DMBA can induce malignant melanomas which resemble the melanomas of man.[235,236] Four of 20 female guinea pigs developed malignant melanomas and subsequent metastases after receiving 50 to 100 applications of 0.5 mg DMBA in benzene.[236] Twenty-six male guinea pigs developed only alopecia and hyperpigmentation at the site of DMBA application; one male developed a non-metastasizing subcutaneous liposarcoma.

As a tumor initiator, DMBA is greater than BaP in potency. Slaga and coworkers[233] provided dose-response data in female CD-1 mice for tumor

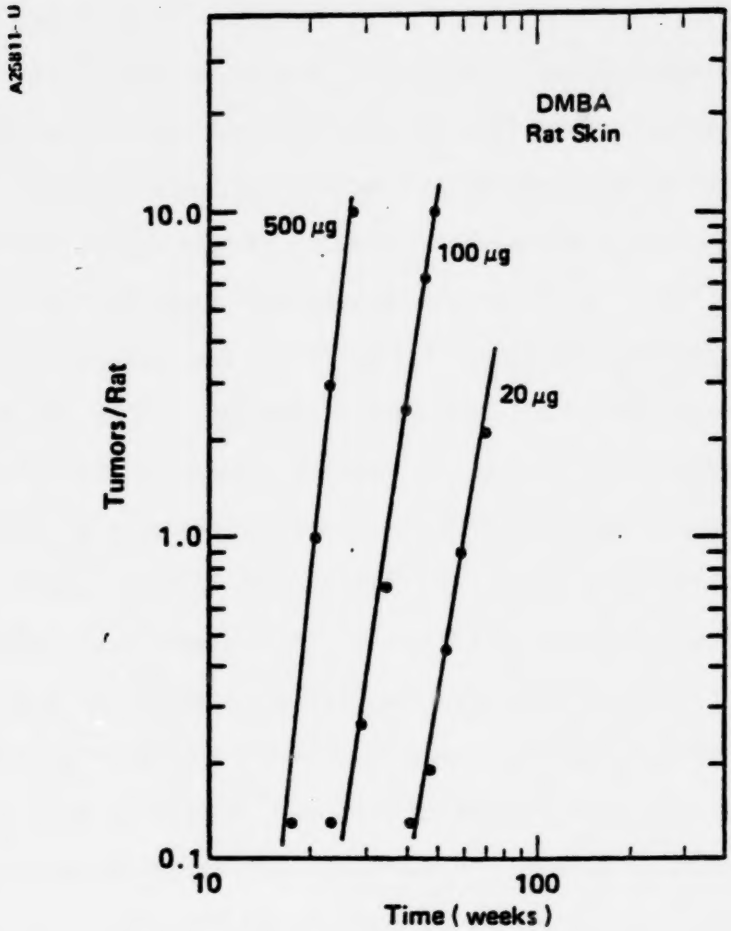

Figure 6-13. Incidence of cancer per rat after weekly topical application of either 20, 100, or 500 µg DMBA in 1.0 ml acetone.[142]

production by single dose DMBA application followed by twice weekly treatment with 0.25 percent croton oil. A single dose of either 0.5, 5, 10, 20, 100, or 200 nmoles DMBA produced 0.4, 2.3, 6.0, 5.5, 6.2, and 16 papillomas per mouse, respectively, at 24 weeks. At the lowest DMBA dosage (0.5 nmoles), 31 percent of treated mice bore tumors after 34 weeks. With higher doses of DMBA the tumor incidence ranged from greater than 60 percent (5 nmoles) to 100 percent (200 nmoles). When TPA, the active phorbol ester ingredient in croton oil, was used as the promoter (17 nmoles) instead of croton oil, both tumor incidence and tumor yield were increased. In this study, the effect of higher DMBA doses (i.e., 10, 20, or 100 nmoles) was primarily a shortening of the tumor latency period since tumor incidences and the average number of tumors per mouse were similar after 24 weeks. However, at the 200 nmole dose level DMBA produced more than twice the number of tumors as the 100 nmole treatment. The authors suggested that by increasing the dose of DMBA to 200 nmoles a promoting stimulus is superimposed upon initiation while at the same time increasing the number of effectively initiated sites. Therefore, it was implied that the magnitude of an initiating stimulus can be evaluated in terms of the number of tumors eventually produced and their latency period, rather than by the final tumor incidence. It must be kept in mind, however, that the effectiveness of DMBA in the two-stage skin carcinogenesis system is influenced by species susceptibility and factors which modify the promotion process (diet, hormonal status).[237] These principles probably apply as well to most polycyclic carcinogens.

Sarcomas developing at the site of injection are frequently observed with DMBA, much the same as with BaP injection. A single injection of

2.5 mg DMBA produced sarcomas in all of a group of eight female Sprague-Dawley rats. In a similar study, 6 μmoles of DMBA (administered as 30 injections of 0.2 μmoles each on alternate days) produced a 100 percent incidence of sarcoma in 10 female Sprague-Dawley rats. The same treatment employing BaP instead of DMBA led to essentially identical results. Injections of principal DMBA hydroxylated metabolites or its K-region epoxide, on the other hand, were much less effective in producing sarcomas. In C57 black mice, 10 weekly injections of one mg DMBA produced local tumors (sarcomas and carcinomas) in four of six males and three of five females. Monohydroxymethyl derivatives of DMBA were active as carcinogens, but less potent than the parent compound.

6.4.3.4 3-Methylcholanthrene--As with DMBA, MCA is a potent carcinogen which is produced synthetically for use in the laboratory. The action of MCA as a complete carcinogen in mouse skin was recently investigated by Burki and coworkers.[238] A single application of 3 μmoles MCA produced 16 tumors in 18 BALB/c male mice after 57 weeks. In a subsequent series of experiments, repeated applications of 1.5 to 3 μmoles MCA twice weekly for three or 17 weeks induced larger numbers of skin tumors in nearly all animals. Nevertheless, MCA is not as potent a skin carcinogen as either BaP or DMBA. However, the incidence and number of tumors produced by MCA could be increased by the administration of trichloropropene oxide, an epoxide hydrase inhibitor and scavenger of glutathione. Despite this effect, which implicates the involvement of a carcinogenic epoxide intermediate by increasing its half-life, the K-region epoxide of MCA (MCA-11,12-oxide) was much less effective than the parent compound. It is possible, however, that such epoxides are too unstable to pass through the cornified layer in the skin to reach the dividing cells in the epidermis.

The skin tumor initiating activity of MCA is comparable to that of BaP when TPA is used as a promoting agent. In CD-1 female mice, a 50 nmole application of MCA followed by twice weekly applications of 10 μg TPA produced an average of 1.9 papillomas per mouse after 30 weeks.[239] The tumor incidence was greater than 50 percent. Pretreatment of mice with TCPO slightly increased the yield of MCA-induced papillomas per mouse, again suggesting the involvement of an epoxide intermediate.

Cavalieri and coworkers[240] have demonstrated an isotope effect for skin tumor induction by MCA. Deuteration at positions C-1 and C-5 diminished the tumorigenic activity of MCA (0.2 μmoles twice weekly for 20 weeks) on the skin of female Swiss mice. It was suggested that metabolic activation and binding of MCA may involve C-1 hydroxylation followed by binding to critical intracellular sites. In support of this hypothesis, it was noted that 1-hydroxy-MCA and MCA-1-one are sarcomagenic for mice by subcutaneous injection.

Subcutaneous injection of relatively high doses of MCA produced tumors in high yields at the injection site in C57 black mice.[241] Three weekly injections of 1 mg MCA produced sarcomas in all of a group of 10 male mice. Ten of 11 female mice developed local tumors after receiving 10 injections of 1 mg MCA each.

6.4.3.5 Dibenz[a,h]anthracene--DBahA was the first pure chemical shown to produce tumors in animals. A skin tumor incidence of 11.7 percent resulted within 28 weeks after female CD-1 mice received ten daily applications of DBahA at 1.0 mg (3.6 μmoles) each.[239] Other investigators have clearly established the ability of DBahA to act as a complete carcinogen in mouse skin; tumor production by DBahA is dose-related and similar in potency to BaP. The K-region epoxide (see Section 6.4.2) of DBahA (DBahA-5,6-epoxide) produced about the same tumor response as the parent compound in female CD-1 mice.[239]

DBahA was shown to be as effective a tumor initiator in mouse skin as BaP.[239] A single application of 200 nmoles DBahA followed by twice weekly applications of 10 μg TPA for 30 weeks yielded an average of four papillomas per animal in about 90 percent of a group of 30 CD-1 female mice. As little as 0.02 μg (0.07 nmoles) DBahA can initiate skin carcinogenesis in mice.[242]

By subcutaneous injection, DBahA has produced sarcomas and carcinomas at the injection site in C57 black mice. Ten weekly injections of 1 mg DBahA produced tumors in all of a group of 20 male mice and in 17 of 19 female mice. The K-region epoxide of DBahA produced tumors in mice of both sexes, but was not as potent as the parent compound.

Beuning and coworkers[532] recently demonstrated that DBahA 3,4-dihydrodiol is a proximate carcinogen when applied to mouse skin and when injected in newborn mice, as would be predicted by the bay region theory (see Section 6.4.2). The authors established that of the three possible dihydrodiol metabolites of DBahA (1,2-, 5,6-, and 3,4-dihydrodiol) only DBahA 3,4-dihydrodiol showed significant tumorigenic activity. In addition, 42 percent of male newborn mice injected with DBahA 3,4-dihydrodiol (420 nmole total) developed liver tumors whereas all mice treated with DBahA, DBahA 1,2- and 5,6-dihydrodiol showed normal liver histology.

6.4.3.6 Dibenzo[a,h]pyrene--Skin application of DBahP was first associated with the development of epitheliomas in 1939.[208] More recently, Hoffmann and

Wynder[243] reported strong carcinogenic activity on mouse skin when applied for one year, thrice weekly, in 0.05 percent and 0.1 percent concentrations. DBahP was also a strong tumor initiator. Both the carcinogenic and tumor-initiating activity of DBahP, however, were less than that of BaP.

Several investigators have demonstrated that DBahP will consistently induce sarcomas when injected in rats or mice.[208] A total administration of 1.8 mg DBahP produced 34 sarcomas among 35 male mice; only one of ten female mice developed a sarcoma, however.[225]

The reduced carcinogenic activity relative to BaP for DBahP (and other related POM) may be due to the very large decrease in solubility resulting from the addition of another benzene ring. A decreased solubility would imply a reduced ability to metabolize (and presumably activate) these compounds, although confirming biochemical studies are not available. Moreover, it is difficult to compare compounds such as the dibenzopyrenes to the more potent carcinogens (e.g., BaP, DMBA), since high doses over long periods are used to assess tumorigenicity. In such cases, purity of the test compound and saturation of metabolic pathways may confuse the interpretation of results.

6.4.3.7 Dibenzo[a,i]pyrene--Hoffmann and Wynder[243] have established the carcinogenicity of DBaiP in mouse skin. Among 20 female Swiss Albino mice painted three times a week for one year with a 0.1 percent DBaiP solution, 16 animals developed 15 epitheliomas and 29 papillomas. With a 0.05 percent DBaiP solution, the results were essentially the same (13 epitheliomas, 28 papillomas). The tumor latency period for DBaiP-induced skin tumors was longer than the latency period with BaP. DBaiP was also an active tumor initiator, but less effective than either DBahP or BaP.

Large numbers of sarcomas in mice and hamsters are produced by subcutaneous and/or intramuscular injections of DBaiP.[208] Single subcutaneous injections of only 0.5 mg DBaiP into the groin of C57 mice produced local sarcomas in 98 percent of the animals in 24 weeks.

6.4.3.8 Dibenzo[c,g]carbazole--Skin tumors have been produced in mice by painting with DBcgC, although a high mortality associated with the treatment hampers the interpretation of results. Studies summarized by the International Agency for Research on Cancer[208] reported that repeated applications of DBcgC in solutions of 0.1 to 0.5 percent induced papillomas and carcinomas in high percentages.

Local sarcomas have been induced by DBcgC in as many as 100 percent of mice receiving injections. In single doses as low as 0.2 mg, DBcgC produced 17 sarcomas among 20 mice within 40 weeks.[208] Depending on vehicle of administration (lard, olive oil, sesame oil) and strain of mouse employed (C3H, A, C), the incidence of DBcgC-induced sarcomas by a single 0.2 mg injection was as follows: 17/93 (C3H mice, lard); 72/133 (A mice, sesame oil); 20/27 (A mice, olive oil); 5/19 (C3H mice, lard); 22/41 (C3H mice, sesame oil); 46/67 (C3H mice, sesame oil).

6.4.3.9 Benzo[c]phenanthrene--Benzo[c]phenanthrene and several of its monomethyl derivatives were tested for carcinogenicity by skin painting at high doses and subcutaneous injection in C3H mice.[244] Twice weekly application of a 0.5 percent solution in acetone to the shaved backs of 20 mice produced three carcinomas and two sarcomas over the 638 day experimental period. Single subcutaneous injections of 5 mg benzo[c]phenanthrene to 20 mice resulted in three sarcomas. Markedly enhanced tumorigenic potency was evident for the 4-methyl and 5-methyl derivatives of benzo[c]phenanthrene when applied to the skin surface, and for the 5-methyl derivative when injected subcutaneously.

6.4.3.10 Benzo[b]fluoranthene--BbF is a common pyrolysis product found in automobile exhaust, polluted air, and cigarette smoke.[208] In 1959, Wynder and Hoffmann[245] reported that BbF had relatively high carcinogenic activity when applied repeatedly to the skin of female Swiss mice. Among 20 mice receiving thrice weekly applications of BbF (0.5 percent in acetone), all of the animals developed papillomas within five months, and 19 of the 20 mice developed carcinomas after eight months. None of the mice survived beyond eight months. At a dose of 0.1 percent, BbF produced papillomas in 13 animals and carcinomas in 17 animals with 12 months. At 0.01 percent, the lowest dose tested, five of the ten surviving mice after 14 months developed papillomas. By comparison, BaP at the same 0.01 percent dose level caused carcinomas in 17 of 20 mice after 12 months of treatment.

In later studies by Van Duuren and coworkers,[246] BbF was shown to be a potent tumor initiator. A single application of 1 mg BbF in acetone to 20 female KCR/Ha Swiss mice, followed by thrice weekly applications of 25 μg croton resin, produced 18 mice with papillomas and five mice with carcinomas within 63 weeks. The same dose of BbF, followed with no promoting agent, yielded no tumors. By comparison, a 150 μg dose of BaP followed by the same croton resin treatment produced 20 mice with papillomas and six mice with carcinomas over the same time period. The tumor initiating potency of BbF in this test system was slightly greater than for chrysene.

Three subcutaneous injections of 0.6 mg BbF given over two months produced local sarcomas in 8 of 16 males (average latency 130 days) and 10 of

14 females (average latency 133 days).[208,225] The sarcomagenic potency of BbF was considered to be not much less than for BaP, especially in females.

6.4.3.11 Benzo[j]fluoranthene--Little biological information is available concerning BjF, despite the fact that it is a pyrolysis product found in automobile exhaust, polluted air, and cigarette smoke. Nevertheless, Wynder and Hoffmann[245] reported in 1959 that BjF produced carcinomas on the skin of mice. Among 20 female Swiss mice receiving thrice weekly applications of BjF (0.5 percent in acetone), 19 animals developed papillomas and carcinomas within seven months. None of the mice survived beyond seven months. At a dose level of 0.19 percent in acetone, BjF produced papillomas in 14 mice and carcinomas in all 20 mice within nine months.

6.4.4 Respiratory Tract Carcinogenesis

Until recently, the investigation of POM-induced carcinogenesis in tissues of the respiratory tract has been hindered by the lack of a reliable animal model system. Nevertheless, many compounds of the POM class, depending upon the assay system, display marked carcinogenic activity in the respiratory tissues. However, the carcinogenic potency of a polycyclic compound for the pulmonary tissues may not necessarily parallel its activity either as a complete carcinogen on mouse and hamster epidermis, as a tumor initiator on mouse skin, or as a sarcomagenic agent. Nevertheless, bioassay data can serve as guidelines for the prevention of neoplastic diseases in man in at least two ways: (1) the carcinogenic potential of a single chemical can be assessed by examining its effects in multiple animal systems with comparison of target tissues, and by various routes of administration; or (2) a series of related chemicals can be compared within the same bioassay system and ranked according to their tumorigenic potency based on dose-response relationships.

An examination of comparative carcinogenicities within the same tumor system can provide valuable insight concerning relative risks of various POM. By single intravenous injection of about 0.25 mg of aqueous dispersions of polycyclic hydrocarbons to strain A mice, a direct comparison of carcinogenic potency was possible (Table 6-19).[247] In this test system, MCA displayed the greatest lung tumor-forming capability; DBahA followed closely in activity, with DBcgC and BaP being considerably less potent. These results demonstrate the variability in tissue-specific response to POM, since BaP is a more active carcinogen in skin and as a transforming agent *in vitro*.

Intratracheal instillation of POM to Syrian golden hamsters[188,248,249] has become widely utilized for the conduct of dose-response studies for pulmonary carcinogenesis. Several studies are summarized in Table 6-20 and indicate that: (1) dose-response relationships are clearly evident, and (2) the co-administration of carrier particles such as Fe_2O_3 (i.e., with BaP) can markedly increase tumor incidence, depending upon the physical characteristics of the particle. Since environmental exposures to BaP occur in conjunction with particulate material in air, this effect may be particularly relevant to human situations. In addition, increasing doses of carcinogen generally decreased the tumor latency period.

Recently, preliminary results have become available on a study to establish temporal and dose-response relationships for BaP carcinogenesis in the hamster respiratory tract.[142] Weekly intratracheal instillations of BaP (2.0, 1.0, 0.5, or 0.25 mg) with or without an equivalent amount of Fe_2O_3 were conducted for 15 or 30 weeks. Initial results, based on gross observation only, indicated that the incidence of respiratory nodule formation was

Table 6-19. COMPARATIVE CARCINOGENICITY OF POLYCYCLIC HYDROCARBONS AND RELATED COMPOUNDS, MEASURED BY INDUCTION OF LUNG TUMORS (LT) IN STRAIN A MICE[a][247]

Compound	Dose, μmoles/kg	Mice with LT/ no. of mice	Mean no. LT/mouse	μMoles/kg for 1 LT response
3-Methylcholanthrene, 0.1 mg	15	15/15	11	0.9
3-Methylcholanthrene, 0.5 mg	74	6/6	47	
Dibenz[a,h]anthracene	36	10/10	31	1.0
7H-Dibenzo[c,g]carbazole	38	12/12	5.7	6.0
Benzo[a]pyrene	40	10/10	3.7	9.5
Dibenz[a,j]aceanthrylene	33	9/10	2.7	14
Dibenz[a,h]acridine	36	11/12	2.0	18
8-Methylbenzo[c]phenanthrene	42	6/11	0.7	--
7-Methylbenzo[a]pyrene	38	5/10	0.6	--
5-Methoxy-7-propylbenz[a]anthracene	33	1/10	0.1	--
Benz[a]anthracene	44	2/11	0.2	--
Untreated controls	--	4/19	0.2	--

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[a] Strain A mice, 8-12 weeks old, received single intravenous injection of 0.24 mg of methylcholanthrene in aqueous dispersion and were killed 20 weeks later.

Table 6-20. INDUCTION OF RESPIRATORY TRACT TUMORS IN SYRIAN GOLDEN HAMSTERS BY INTRATRACHEAL INSTILLATION OF POM

Compound	Number of animals	Total dose, mg	Respiratory tumor incidence, %	Reference
BaP	30	3.25[a]	10	250
BaP	30	6.5[a]	13	250
BaP	30	13[a]	30	250
BaP	29	26[a]	86	250
BaP	28	52[a]	93	250
BaP	48	30[b]	15	16
BaP and Fe_2O_3	48	30[b]	71	16
BaP and Fe_2O_3, coated	49	26.1[c]	73	249
BaP and Fe_2O_3, ground	49	27.4[c]	84	249
BaP and Fe_2O_3, mixed	43	26.3[c]	12	249
BaP and gelatin	46	26.4[c]	17	249
BaP and Fe_2O_3	28 (male), 29 (female)	60[d]	60.7 (male), 58.6 (female)	248
BaP and Fe_2O_3	33 (male), 34 (female)	30[d]	66.7 (male), 58.8 (female)	248
BaP and Fe_2O_3	33 (male), 30 (female)	15[d]	30.3 (male), 30.0 (female)	248
BaP and Fe_2O_3	47 (male), 41 (female)	7.5[d]	12.8 (male), 9.8 (female)	248
BaP	32 (male)	30[e]	42.3	251
BaP	28 (female)	30[e]	57.7	251
DB[a,i]P	48	12[f]	75	190
DB[a,i]P	48	8.5[g]	64.6	190
DMBA and Fe_2O_3	46	1.2[h]	43.5	189
DMBA and Fe_2O_3	28	0.85	46.4	189
DBC and Fe_2O_3	35	45[j]	86	252
DBC and Fe_2O_3	45	15[k]	89	252

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[a] Animals treated once weekly for 52 weeks with BaP suspended in 0.9% NaCl solution.
[b] 3 mg BaP administered once weekly for 10 weeks.
[c] Animals received 30 weekly intratracheal instillations.
[d] Animals received 30 weekly instillations of BaP mixed with equal amounts of Fe_2O_3 and suspended in 0.2 ml saline.
[e] Animals received 30 weekly intratracheal instillations of BaP suspended in 0.9% NaCl.
[f] Animals received 12 weekly intratracheal instillations of 1 mg DB[a,i]P suspended in distilled water.
[g] Animals received 17 weekly intratracheal instillations of 0.5 mg DB[a,i]P suspended in distilled water.
[h] Animals received 100 μg DMBA and 100 μg Fe_2O_3 intratracheally once a week for 12 weeks in saline suspensions.
[i] Animals received 50 μg DMBA and 50 μg Fe_2O_3 intratracheally once a week for 17 weeks in saline suspensions.
[j] Animals received 15 weekly instillations of 3 mg DBC with 3 mg Fe_2O_3 and suspended in 0.2 ml saline.
[k] Animals received 30 weekly instillations of 0.5 mg DBC with 0.5 mg Fe_2O_3 and suspended in 0.2 ml saline.

dose-dependent; 0.25 mg BaP with 0.25 Fe_2O_3 given for 15 or 30 weeks produced no nodules, whereas 2.0 mg BaP with 2.0 mg Fe_2O_3 given for 15 or 30 weeks resulted in a tumor incidence of 44 percent and 86 percent, respectively. Dose-response information is depicted in Figure 6-14. Surprisingly, the use of Fe_2O_3 as a carrier for BaP had no apparent effect on the incidence of lung nodule formation (Figure 6-15) in this recent study. While tumor latency periods decreased with increasing BaP weekly dose, no clear trend was obtained in temporal aspects of tumor development between 15 and 30 week treatments at the same dose.

Mohr[540] recently compared several methods of carcinogen administration for the production of respiratory tract tumors in the Syrian hamster. Three different methods were employed for the administration of BaP, instillation, implantation, and inhalation. Whereas both instillation and direct implantation of BaP were very effective in the induction of respiratory tumors, inhalation exposure yielded no tumors. Hamsters were exposed to BaP aerosols of 40-50 mg/m^3 of air for 4.5 hours per day over a period of three to four months and observed for one year. These negative results underscore the need to carefully examine carcinogenic activity of POM under environmentally realistic conditions of exposure if reliable dose-response data are desired.

In addition to the hamster model system, respiratory lesions and carcinomas have been readily induced by POM in rats and mice by various routes of administration. The results of several representative studies are summarized in Table 6-21.

An additional approach used to evaluate the effects of POM on the pulmonary tissue was reported by Flaks and Sims.[253] Pulmonary tissue taken from tumor susceptible female BALB/c mice was incubated for 30 minutes in the

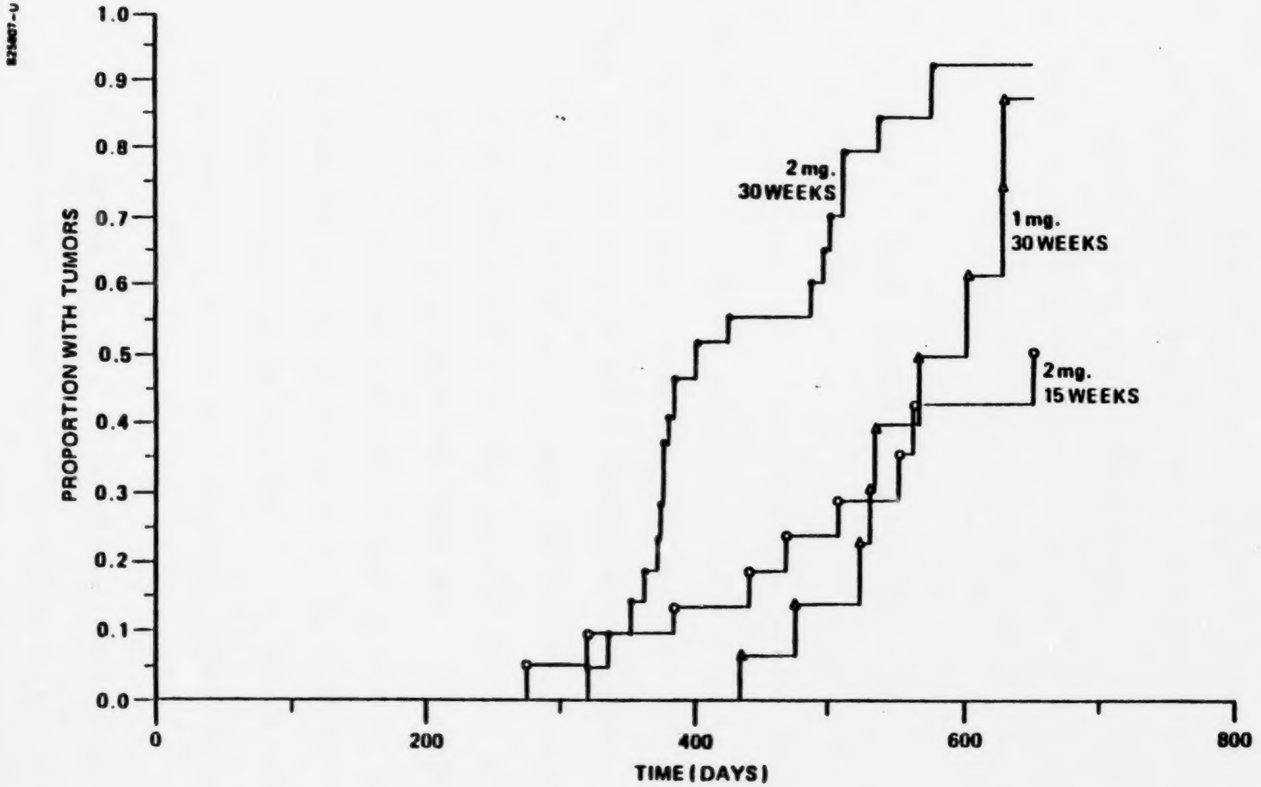

Figure 6-14. Respiratory nodule incidence in hamsters given BaP by intratracheal instillation.[142] (Hamsters were given weekly intratracheal instillations of either 1.0 or 2.0 mg BaP with equivalent amounts of Fe_2O_3 for either 15 or 30 weeks as indicated. Proportion with tumor was calculated from the life table procedure on the basis of tumors found at death. Time was measured from the first BaP dose.)

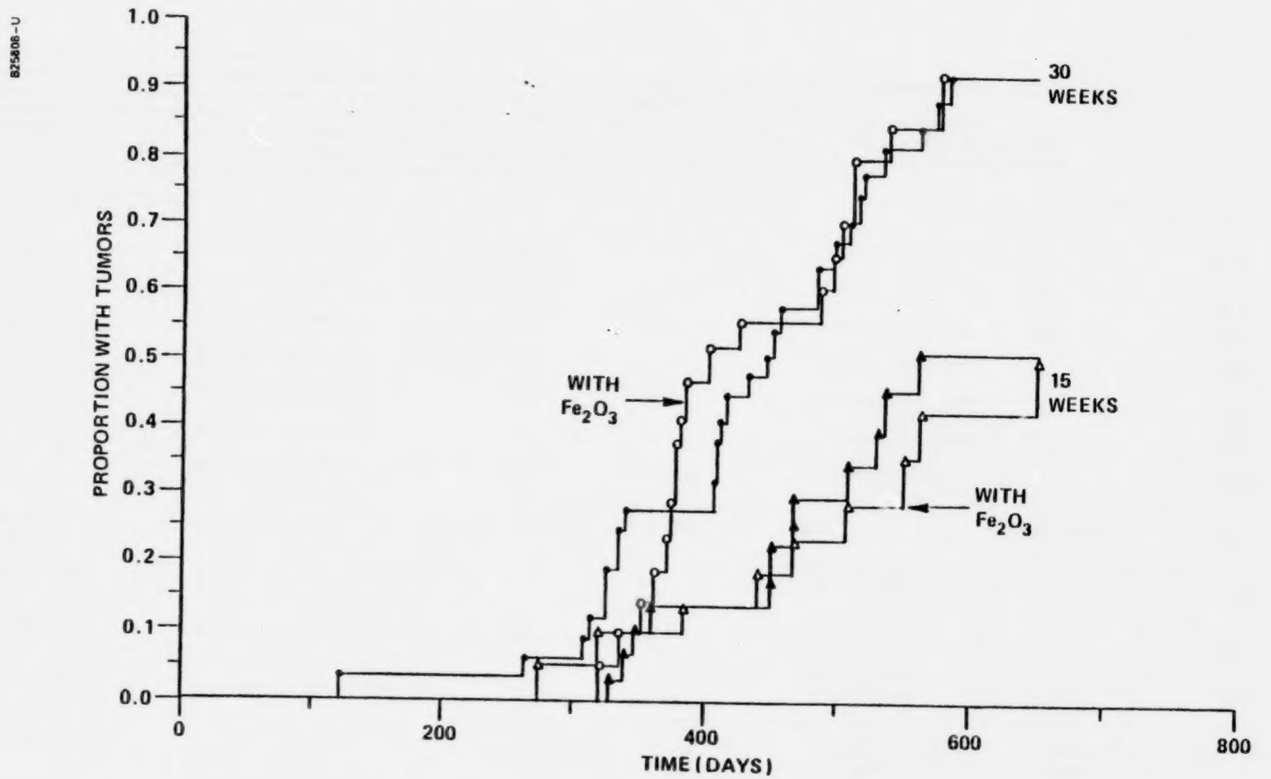

Figure 6-15. Respiratory nodule incidence in hamsters given BaP by intratracheal instillation with and without Fe_2O_3.[142] (Hamsters were given weekly intratracheal instillations of 2.0 mg BaP for either 15 or 30 weeks with and without 2.0 mg ferric oxide (Fe_2O_3). There were 36 hamsters per group and tumors found at death were analyzed by the life table procedure to give the proportion with tumor. Time was measured from the first BaP dose.)

Table 6-21. INDUCTION OF RESPIRATORY TRACT TUMORS IN RATS AND MICE

Compound	Organism	Number of animals	Total dose, mg	Route of administration	Tumor incidence, %	Reference
DMBA and Indian ink	Rat (Wistar and random-bred)	34	2.5[a]	Intratracheal instillation	17.6	260
DMBA and Indian ink	Rat (Wistar and random-bred)	56	6[b]	Intratracheal instillation	35.7	260
DMBA and Indian ink	Rat (Wistar and random-bred)	61	10[c]	Intratracheal instillation	26.2	260
DB[a,h]A	Mouse (DBA/2)	14 (male) 13 (female)	236 (male)[d] 179 (female)[d]	Oral	100 (male)[e] 77 (female)[e]	261
MCA	Rat (Osborne-Mendel)	100	0.005[f]	Pulmonary injection	1[g]	262
MCA	Rat (Osborne-Mendel)	100	0.05[f]	Pulmonary injection	13[g]	262
MCA	Rat (Osborne-Mendel)	100	0.10[f]	Pulmonary injection	27[g]	262
MCA	Rat (Osborne-Mendel)	100	0.20[f]	Pulmonary injection	47[g]	262
MCA	Rat (Osborne-Mendel)	100	0.30[f]	Pulmonary injection	40[g]	262
MCA	Rat (Osborne-Mendel)	100	0.40[f]	Pulmonary injection	51[g]	262
MCA	Rat (Osborne-Mendel)	100	0.50[f]	Pulmonary injection	45[g]	262

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[a] Administered as a single dose with 0.2 mg of Indian ink in 0.2 ml of a colloid protein solution.

[b] Administered as three 2 mg doses at monthly intervals with 0.2 mg of Indian ink in 0.2 ml of a colloid protein solution.

[c] Administered as five 2 mg doses at monthly intervals with 0.2 mg of Indian ink in 0.2 ml of a colloid protein solution.

[d] Administered as an aqueous-olive oil emulsion of DB[a,h]A given in place of drinking water for 237 to 279 days.

[e] Tumors were alveologenic carcinomas; a 100% incidence of pulmonary adenomatosis was also observed.

[f] Administered as a single MCA-containing beeswax pellet placed directly into the lower peripheral segment of the left lung.

[g] Overt squamous cell carcinoma.

presence of 4 μg/ml of DMBA, DBahA, BaP, or one of their related K-region epoxides, and subsequently implanted into isologous mice. Under these conditions, only DMBA showed marked carcinogenic activity.

Squamous cell carcinomas have been induced in rat tracheal grafts by instillation of MCA, BaP, or BaP mixed with Fe_2O_3 particles.[254] Neoplasms were induced by 5 mg of carcinogen after 35 weeks of contact in 3 of 5, 2 of 5, and 2 of 3 tracheal grafts exposed to MCA, BaP, and BaP-Fe_2O_3, respectively. Subsequent studies in the same test system using DMBA in a sustained release formulation established that carcinomas could be induced in tracheal epithelium at doses as low as 40 μg.[255] With this procedure, the lowest dose of BaP tested which produced a carcinoma was 300 μg.[256]

6.4.5 Carcinogenesis in Newborn Mice

The newborn mouse is known to be highly susceptible to carcinogenic POM,[257] presumably because of its large numbers of rapidly dividing cells,[258] and is thus a useful model for carcinogenesis studies. In studies reported by Asahina and coworkers,[259] various fractions of an organic extract of atmospheric particulate pollutants displayed high carcinogenic activity when injected subcutaneously to infant Swiss mice of both sexes. Various combinations of remote tumors (hepatomas, lymphomas, pulmonary adenomas) were produced by the basic, neutral, and aromatic fractions of the benzene soluble organic extract. Three oxyneutral subfractions also produced multiple tumors. These results indicated that a variety of different chemical carcinogens are present in organic extracts of air pollutants in addition to BaP, which is found mainly in aromatic fractions. Walters[263] administered DMBA by single injection to male and female newborn (<24 hours old) BALB/c mice at doses ranging from 0.625 to 40 μg. At the lowest dosage, the incidence of lung tumors was greater than 50 percent in females and greater than 75 percent in males. Subcutaneous injections of 0.005 μg MCA or 0.003 μg DBahA were sufficient to produce fibrosarcomas at the injection site of newborn mice.[264] However, on a mg/kg of body weight basis these doses are comparable to carcinogenic doses in adult animals. Attempts to determine whether the appearance of DMBA-induced lung tumors is more likely at a definite point in the life span of a mouse have not produced conclusive results.[265]

The K-region epoxides of DBahA and MCA were found to be negative for carcinogenic activity in newborn mice, despite reports that the parent hydrocarbons were active at the same dose levels (60 μg).[266] On the other hand, the 3,4-dihydrodiol of DBahA is highly tumorigenic in the newborn mouse,[532] as would be predicted by the "bay region" theory (see Section 6.4.3.5). These results suggested that K-region epoxides may not be the ultimate metabolites involved in POM carcinogenesis as once thought. In support of the contention that a dihydrodiol precusor of the "bay region" diol epoxide may be a proximate carcinogenic metabolite of BaP, Kapitulnik and coworkers[66,68] recently showed that BaP 7,8-dihydrodiol is more carcinogenic than BaP in newborn mice. Moreover, one of the stereoisomeric BaP 7,8-diol-9,10-epoxides, (±)-trans-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-BaP, demonstrated extremely high carcinogenic activity in newborn mice, as would be predicted by the "bay region" theory (see Section 6.4.2). Since the metabolism of BaP results in three known diols, all via epoxide intermediates, it thus appears that the major pathway for carcinogenic activation may be:

$$\text{BaP} \longrightarrow \text{BaP 7,8-oxide} \longrightarrow \text{BaP 7,8-dihydrodiol} \longrightarrow \text{BaP 7,8-diol-9,10-epoxide}$$

which results in formation of the diol epoxide as an ultimate carcinogen.

Wislocki and coworkers[267] recently presented evidence supporting the "bay region" theory with regard to the carcinogenicity of benz[a]anthracene (see Section 6.4.3.2). They demonstrated that benz[a]anthracene-3,4-dihydrodiol possessed about 35 times the activity of the parent hydrocarbon and more than 300 times the activity of the other four isomeric dihydrodiols in producing lung adenomas in the newborn mouse. The "bay region" theory predicts that benz[a]anthracene-3,4-diol-1,2-epoxide would be the reactive ultimate carcinogenic metabolite of benz[a]anthracene. Since the 3,4-dihydrodiol is the metabolic precursor of the 3,4-diol-1,2-epoxide, it acts as a proximate carcinogen according to "bay region" theory predictions.

6.4.6 Tumors in Other Tissues

Mammary cancer induced in rodents by DMBA has become an important model system for the study of human breast cancer.[268,269,270] The demonstration of the production of hormone-responsive mammary tumors in normal female Sprague-Dawley rats by a single intragastric dose of DMBA by Huggins and coworkers[271] in 1961 introduced a simple, yet sensitive, model for the experimental investigation of the biology of mammary tumor growth. A year later, Huggins and coworkers[272] demonstrated that a single intragastric feeding of 20 mg DMBA resulted in the production of mammary cancer or fibroadenoma in 100 percent of the treated animals.

In 1965, Huggins[273] reported that intragastric feeding of DMBA could be substituted with a single intravenous injection of 5 mg DMBA in lipid emulsion for the purpose of producing mammary cancer in rats. By this technique, Huggins successfully induced mammary carcinoma in 1500 consecutive female Sprague-Dawley rats, age 50 days. Tumors were detected by palpation as early as 20 days after DMBA injection. In a study with 90 rats, mammary cancer was detected in 28 to 92 days, with a mean time of appearance of 42.8 ± 11 days. In 1966, Griswold[268] adapted the DMBA-induced mammary cancer technique in rats as a routine for the evaluation of potential anti-cancer agents. Using the Huggins system, Griswold found that tumor regression was produced in DMBA-treated tumor-bearing rats by hypophysectomy or ovariectomy at 90 days after feeding of DMBA and by injection of testosterone propionate beginning 120 days after DMBA feeding.

It is now known that mammary cancer can be induced by DMBA in rats not only by systemic administration but also by local application of the carcinogen to the mammary gland.[274] These tumors were primarily adenocarcinomas of ductal origin and were microscopically evident as early as 30 days after local application of 1 mg DMBA. The minimal dose of DMBA to produce a mammary tumor was 300 μg. It is important to note that in this study hyperplastic alveolar nodules did not form in the mammary glands after local DMBA application, thereby disputing earlier contentions that these "preneoplastic" lesions were a prerequisite to mammary carcinogenesis. Thus, it was demonstrated that transformation to a neoplastic state may occur directly and require no intermediate steps.

In addition to DMBA, ingestion of BaP may also result in mammary tumor formation. Albert and coworkers[142] reported the development of multiple mammary nodules in Lewis rats following a single intragastric feeding of 50 mg BaP. Numerous nodules were present within 50 days of the treatment.

POM-induced cancers of the endocrine target organs are not limited to the mammary gland. Ovarian granulosa cell tumors in mice result from exposure to BaP and MCA.[275] In Wistar rats, a silk thread impregnated with DMBA and inserted in the region of the ovary produced 47 ovarian solid tumors among 121 animals.[276]

In addition to ovarian cancers, DMBA can also produce carcinoma by local application to the pancreas of Sprague-Dawley rats.[277] Less than 1 mg of DMBA placed directly into the pancreas induced adenocarcinoma in eight of ten treated animals within 180 days.

6.4.6.1 Oral exposure to POM--Oral administration of POM frequently results in papillomas and carcinomas in the forestomach of rodents. Numerous studies conducted with BaP, DBahA, and DBcgC given orally to rats, mice, and hamsters have recently been reviewed,[208] and some of these data are summarized in Table 6-22. Tumors of the stomach have also been induced by MCA[279] and DMBA.[280] A single feeding of 3 mg DMBA induced nine stomach papillomas in 18 surviving Swiss mice after 1 year.[280] Ovarian, lymphoid, mammary, and hepatic tumors were also observed. In addition, the tumorigenicity of DMBA in the rat submandibular gland has been repeatedly demonstrated.[281]

Very little work has been done to characterize dose-response relationships for POM-induced tumors by oral administration. The most extensive studies in this area have been conducted by Rigdon and Neal.[282,284] Their studies involved gastric tumors (papillomas and squamous cell carcinomas), pulmonary adenomas, and leukemia in CFW mice fed BaP. Small groups of mice were fed BaP at various concentrations in their chow, and for different periods of time, up to a maximum of 197 days. In some groups, an observation period of from 104 to 114 days followed the treatment period. The results of Rigdon and Neal[283] are summarized in Table 6-23. The authors noted that

Table 6-22. CARCINOGENICITY OF POM BY ORAL ADMINISTRATION TO VARIOUS MAMMALS[208]

Compound	Species	Dose	Route of administration	Effects
BaP	Mouse	0.2 mg in PEG[a]	Intragastric	14 tumors of the forestomach in 5 animals out of 11
	Rat (Sprague-Dawley; age 105 days)	2.5 mg per day	Oral	Papillomas developed in the esophagus and forestomach in 3 out of 40 animals
	Hamster	2-5 mg bi-weekly	Intragastric	5 stomach papillomas in 67 animals treated for 1-5 months; 7 papillomas and 2 carcinomas in 18 animals treated for 6-9 months; 5 papillomas in 8 animals treated for 10-11 months
	Hamster	500 ppm	Dietary (4 days per week for up to 14 months)	12 tumors (2 esophagus, 8 forestomach, 2 intestinal) in 8 animals
DB[a,h]A	Mouse	9-19 mg (total dose)	Dietary (5-7 months)	Forestomach tumors in 7 of 22 survivors after one year; one tumor was a carcinoma
	Mouse (A backcross)	0.4 mg per day	Oil emulsion (drinking water replacement)	11 papillomas of the forestomach in 20 animals within 406 days
	Mouse (DBA/2)	0.76-0.85 mg per day	Oil emulsion (drinking water replacement)	Pulmonary adenomatosis in all 27 survivors at 200 days; 24 animals had alveologenic carcinomas; 16 had hemangio-endotheliomas; 12 of 13 females had mammary carcinomas; 2 pulmonary adenomatoses seen among 35 controls

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[a] Polyethylene glycol

Table 6-22 (continued). CARCINOGENICITY OF POM BY ORAL ADMINISTRATION TO VARIOUS MAMMALS[208]

Compound	Species	Dose	Route of administration	Effects
	Mouse (Swiss, male)	1.5 mg in PEG[a]	Oral (single dose)	Papillomas of the forestomach in 2 out of 42 animals within 30 weeks
	Mouse (BALB/c, female)	15 mg total dose in almond oil	Intragastric (twice weekly for 15 weeks)	Mammary carcinomas in one out of 20 intact animals and 13 out of 24 pseudo-pregnant animals
DB[c,g]C	Mouse (CBA or Strong A strain)	7-23 mg total dose in arachis oil	Oral (twice weekly)	Among 55 mice surviving 17 to 59 weeks, 64% had papillomas and 13% had squamous carcinomas of the forestomach; 19 animals had benign hepatomas and another 19 animals had malignant hepatomas; pulmonary adenomas observed in all Strong A mice and none of the CBA mice

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[a] Polyethylene glycol

Table 6-23. GASTRIC TUMORS IN MICE FED BENZO[a]PYRENE[283]

No. of mice	Age in days	Mg BaP per g food	No. of days fed BaP	Total amt. BaP (mg) fed per mouse	No. of days fed lab. chow	Age killed in days	% Mice with gastric tumors
25	30	0.001	110	0.48	0	140	0
24	30	0.01	110	4.48	0	140	0
23	116	0.02	110	8.88	0	226	5
37	33-67	0.03	110	13.32	0	143-177	0
40	33-101	0.04	110	17.76	0	143-211	2.5
40	31-71	0.045	110	19.80	0	141-183	10.0
34	17-22	0.05	107-197	21.4- 29.4	0	124-219	70.0
23	20-24	0.10	98-122	39.2- 48.8	0	118-146	82.0
73	18-20	0.25	70-165	70.0-165.0	0	88-185	90.0
10	49	0.25	1	1.0	105	155	0
9	56	0.25	2	2.0	104	162	11.0
10	49	0.25	4	4.37	102	155	10.0
9	62	0.25	5	5.13	101	168	44.0
10	49	0.25	7	6.10	99	155	30.0
26	91	0.25	30	30.0	77	198	100.0
10	74	0.10	7	2.8	101	182	0
18	48	0.10	30	12.0	78	156	67.0
33	98-180	5.0	1	12.0	103-113	202-294	50.0

among 289 mice fed a control ration, none developed gastric tumors. As would be expected, the frequency of BaP-induced gastric tumors was dependent both on the concentration of BaP in the food, and the number of days it was fed.

The role of genetic factors in determining tumor response to orally administered BaP in mice was subsequently explored by Rigdon and Neal.[284] Since it is known that the development of pulmonary adenomas in certain strains of mice is genetically influenced, a comparison was made between the incidence of pulmonary adenomas and gastric tumors and/or leukemia in CFW Swiss mice. A ration containing 0.25 mg of BaP per gram of food was fed to a total of 108 mice for 80 to 140 days. Their results are summarized in Table 6-24. A large number of mice developed leukemia, even though the rate of spontaneous occurrence in this strain of mouse was considered to be very low. Among leukemic mice a statistically significant increase in pulmonary tumor formation was observed, whereas a negative statistical correlation with gastric tumors was seen in this group. Furthermore, there was no relationship between the occurrence of pulmonary adenomas and gastric tumors in mice without leukemia.

6.4.7 Carcinogenicity of POM Mixtures

In the environment, man is unlikely to come in contact with only a single POM, regardless of the route of exposure. Instead, POM occur as complex mixtures in all environmental media. Despite this generally accepted fact, very few studies have been conducted on the carcinogenicity of defined POM mixtures. The importance of studying the effects of POM in mixtures is that it allows for the expression of possible cocarcinogenic, anticarcinogenic, and tumor-promoting effects.

Table 6-24. RELATIONSHIP OF LEUKEMIA TO LUNG AND STOMACH TUMORS IN 108 MICE FED BENZO[a]PYRENE.284

Total no. of mice	Number of mice with			
	Leukemia	Lung tumors	15 or more lung tumors	Stomach tumors
9	9	0	0	0
23	23	23	12	0
5	5	5	4	5
3	3	0	0	3
7	0	7	3	0
17	0	17	1	17
44	0	0	0	44
108	40	52	20	69

Among the most relevant studies conducted on the effects of POM mixtures were those concerned with the carcinogenic components of automotive engine exhaust. Pfeiffer[285,286] treated groups of 100 female NMRI mice with single subcutaneous injections of a mixture containing 10 noncarcinogenic POM, in addition to BaP and/or DBahA. The treatment combinations and dosages are summarized in Table 6-25. As the results depicted in Table 6-26 indicate, increases in tumor incidence could be attributed to the presence of increased amounts of BaP and of DBahA. It is noteworthy that, at the lower dosages, DBahA was more effective in producing tumors at the injection site than was BaP. Moreover, no effect of the 10 noncarcinogens on tumorigenic response was evident. Probit analysis of tumor incidence data indicated that the tumorigenic response from application of all 12 POM was attributable solely to DBahA.

Similar studies intended to reveal carcinogenic interactions among POM found in automobile exhaust were conducted by Schmähl and coworkers.[287] Eleven POM were selected for their experiments, and various combinations were applied to the skin of NMRI mice in a proportion based on their respective weights in automobile exhaust (Table 6-27). Animals received twice weekly treatments for life (or until a carcinoma developed). Their results (Table 6-28) indicated that a mixture of carcinogenic POM was more effective than BaP alone, and that the whole mixture (carcinogenic plus noncarcinogenic POM) was not significantly more effective than the carcinogenic POM group alone. Thus, the carcinogenic effects observed were solely attributable to the carcinogenic components of the mixture.

Early studies have focused on the carcinogenic activity of simple combinations of two POM, each having different carcinogenic potency when administered alone.[288,289] Compounds were administered by subcutaneous injections to

Table 6-25. Classification of Test Groups[285]

A			B		
	dose (µg)	substance		dose (µg)	substance
A_1	3.12	benzo(a)pyrene	B_1	2.35	dibenz(a,h)anthracene
A_2	6.25		B_2	4.7	
A_3	12.5		B_3	9.3	
A_4	25.0		B_4	18.7	
A_5	50.0		B_5	37.5	
A_6	100.0		B_6	75.0	

C Substance	C_1 dose (µg)	C_2 dose (µg)	C_3 dose (µg)	C_4 dose (µg)	C_5 dose (µg)	C_6 dose (µg)
benzo(e)pyrene	2.15	4.3	8.75	17.5	35.5	70.0
benzo(a)anthracene	3.125	6.25	12.5	25.0	50.0	100.0
phenanthrene	125.0	250.0	500.0	1000.0	2000.0	4000.0
anthracene	31.25	62.5	125.0	250.0	500.0	1000.0
pyrene	65.1	131.2	262.5	525.0	1050.0	2100.0
fluoranthene	28.1	56.25	112.5	225.0	450.0	900.0
chrysene	3.125	6.25	12.5	25.0	50.0	100.0
perylene	0.2	0.4	0.87	1.75	3.5	7.0
benzo(ghi)perylene	12.8	25.6	51.25	102.5	205.0	410.0
coronene	3.125	6.25	12.5	25.0	50.0	100.0

D		E	
D_1	$A_1 + B_1$	E_1	$C_1 + D_1$
D_2	$A_2 + B_2$	E_2	$C_2 + D_2$
D_3	$A_3 + B_3$	E_3	$C_3 + D_3$
D_4	$A_4 + B_4$	E_4	$C_4 + D_4$
D_5	$A_5 + B_5$	E_5	$C_5 + D_5$
D_6	$A_6 + B_6$	E_6	$C_6 + D_6$

Table 6-26. Tumor Incidence Resulting, by the End of the 114th Week, from a Single Subcataneous Application of Test Substances[285]

BaP Group (A)		DBahA Group (B)		BaP + DBahA Group (D)	10 PAH[a] Group (C)	12 PAH Group (E)
Dose (µg)	No. of Tumors	Dose (µg)	No. of Tumors	No. of Tumors	No. of Tumors	No. of Tumors
3.12	9	2.35	37	48	6	41
6.25	35	4.7	39	44	8	55
12.5	51	9.3	44	61	6	61
25.0	57	18.7	56	68	4	72
50.0	77	37.5	65	69	13	68
100.0	83	75.0	69	79	5	82

[a] PAH, polycyclic aromatic hydrocarbon

Table 6-27. Doses (μg) Applied in Dermal Administration Experiments, in Relation to Benzo(a)pyrene[287]

Controls				
Acetone	as solvent			
Benzo(a)pyrene	1.0	1.7	3.0	
C PAH[a]				
Benzo(a)pyrene	1.0	1.7	3.0	
Dibenz(a,h)anthracene	0.7	1.2	2.1	
Benzo(a)anthracene	1.4	2.4	4.2	
Benzo(b)fluoranthene	0.9	1.5	2.7	
total	4.0	6.8	12.0	
NC PAH				
(Benzo(a)pyrene	1.0	3.0	9.0	27.0)
Phenanthrene	27.0	81.0	243.0	729.0
Anthracene	8.5	25.5	76.5	229.5
Fluoranthene	10.8	32.4	97.2	291.6
Pyrene	13.8	41.4	124.2	372.6
Chrysene	1.2	3.6	10.8	32.4
Benzo(e)pyrene	0.6	1.8	5.4	16.2
Benzo(ghi)perylene	3.1	9.3	27.9	83.7
total	65.0	195.0	585.0	1755.0
C PAH + NC PAH				
(Benzo(a)pyrene	1.0	1.7	3.0)	
Total C PAH	4.0	6.8	12.0	
Total NC PAH	65.0	110.5	195.0	
Total C PAH + NC PAH	69.0	117.3	207.0	

Relation of C PAH:NC PAH is constantly 1:16.25

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[a] PAH, polycyclic aromatic hydrocarbon

Table 6-28. Findings at the Site of Application of PAH to Mouse Skin[a] [287]

Application	Single dose ug	Initial no. of animals	Effective no. of animals	Histological diagnosis at the site of application							
				negative abs.	negative %	papilloma abs.	papilloma %	carcinoma abs.	carcinoma %	sarcoma abs.	sarcoma %
Solvent	-	100	81	80	99	-	-	-	-	1	1
BaP	1.0	100	77	66	86	1	1	10	13	-	-
BaP	1.7	100	88	63	72	-	-	25	28	-	-
BaP	3.0	100	81	36	44	2	3	43	53	-	-
C PAH	4.0	100	81	52	64	4	5	25	31	-	-
C PAH	6.8	100	88	31	35	3	3	53	60	1	1
C PAH	12.0	100	90	25	28	1	1	63	70	1	1
NC PAH	65.0	100	85	84	99	-	-	1	1	-	-
NC PAH	195.0	100	84	84	100	-	-	-	-	-	-
NC PAH	585.0	100	88	87	99	-	-	1	1	-	-
NC PAH	1755.0	100	86	70	81	-	-	15	17	1	1
C PAH + NC PAH	69.0	100	89	43	48	1	1	44	49	1	1
C PAH + NC PAH	117.3	100	93	36	39	2	2	54	58	1	1
C PAH + NC PAH	207.0	100	93	28	30	1	1	64	69	-	-

[a] The decimal points have been rounded off; therefore, the sum of % values will not always be equivalent to 100%. PAH = polycyclic aromatic hydrocarbon.

C57 black mice, and the resulting incidence of sarcomas used as the index of carcinogenic activity. The combination of benz[a]anthracene and chrysene produced a tumor response which was more than additive; the combination of benz[a]anthracene and DBahA produced less than additive effects; the combination of DBahA and MCA produced a summation of response. When a moderately active carcinogen (benz[a]anthracene) was combined with a powerful carcinogen, the results varied from summation effects (with MCA) to no-less-than-additive response (with BaP or DBahA). The combination of a strong carcinogen (DBahA) with a weak carcinogen (chrysene) produced no summation, and the combination of strong carcinogen (DBahA) with a noncarcinogen (anthracene, phenanthrene) had no effect on tumor yield.

6.4.8 *In Vitro* Carcinogenesis Studies

Bioassay systems to study the carcinogenic effects of chemicals *in vitro* are desirable because of their relatively low cost and rapid generation of results in comparison to *in vivo* bioassays. Cell culture systems for the study of chemical carcinogenesis are still evolving, and a highly reproducible *in vitro* assay system is not yet available.

The published literature regarding chemical carcinogenesis in cultures is vast, despite the fact that systematic studies were not begun until the early 1960's due to the lack of a reproducible transformation assay. It was first demonstrated by Berwald and Sachs[290] in 1963 that polycyclic hydrocarbons (MCA, BaP) could cause the direct morphological transformation of hamster embryo cells in culture with a low rate of spontaneous transformation in untreated cells. Transformed colonies have growth characteristics visually distinct from normal colonies and are readily seen above a background of normal cells. This assay can therefore be easily used as a screen to compare carcinogenic activity of suspect compounds. A common feature of these, and nearly all, transformed cells is that they give rise to fibrosarcomas upon inoculation into immunosuppressed animals. In addition to hamster embryo cells, malignant transformation has been demonstrated in organ cultures, liver cell cultures, fibroblastic cells derived from mouse ventral prostate, 3T3 cell lines derived from mouse embryo cells, and various types of epithelial cells from animals.[291-297] In general, these test systems are effective with much lower quantities of test material than in bacterial mutagenicity assays. However, the very low numbers of transformed colonies which are usually obtained, even with potent carcinogens, require a careful evaluation of negative control data for the occurrence of spontaneous transformation. In addition, the possibility of contamination of test compounds must always be considered when positive results are obtained.

Early reports by Berwald and Sachs[298] and Dipaolo and Donovan[299] described alterations in hamster embryo cells induced by BaP, DMBA, and MCA which could be used as indicators of a change from a normal to neoplastic state. The compounds were applied to cells in culture either dissolved in paraffin and impregnated on filter disks or as a colloidal suspension in growth medium. Following marked cytotoxicity, foci of transformed cells developed which displayed continuous proliferation _in vitro_, chromosomal abnormalities, and the ability to grow indefinitely in culture. In addition, these transformed mass cultures, when transplanted to 4- to 6-week old hamsters, continued to grow and form tumors. A good correlation was obtained between _in vitro_ carcinogenicity of a polycyclic hydrocarbon and the number of transformed clones they produced. The maximum rate of cell transformation in these studies was 25.6 percent in surviving cells, obtained by treatment with 10 μg/ml of BaP for 6 days. BaP treatment at 1 μg/ml for 6 days produced 19.9 percent transformation in surviving cells. Further data indicating the activity of several polycyclic carcinogens and their derivatives are summarized in Table 6-29. It is significant to note that the K-region epoxides of DBahA and MCA are more active in the production of malignant transformation in hamster embryo cells than the parent hydrocarbons or the corresponding K-region phenols.[300,301] Although these results confirm the view that metabolism is necessary for carcinogenic activity, they conflict with data generated _in vivo_ which indicate that K-region epoxides of polycyclic carcinogens are less active than the parent compound in various species. A possible reason for the lack of correlation is the relative instability of K-region epoxides as compared to the parent hydrocarbon when applied to the skin. It is likely that _in vivo_ far less of the reactive K-region epoxide can survive passage through the skin to reach the basal cell layer. Several investigators have also made it evident that the toxicity and transforming activity of POM are dissociable and occur by different processes,[302,303] with the toxicity being due to random alkylation of nucleophilic regions within the cell. However, when hamster embryo cells are pretreated with weak chemical carcinogens which can induce microsomal enzyme activity (e.g., benz[a]anthracene, methyl methanesulfonate, ethyl methanesulfonate) before the addition of a potent carcinogen (e.g., MCA, BaP, DMBA), transformation may be considerably enhanced.[303,304]

As a prescreen for chemical carcinogens, cell transformation _in vitro_ may be one of the most sensitive techniques available. Pienta and coworkers[305] reported that 90 percent (54/60) of the carcinogens they tested transformed hamster embryo cells _in vitro_, whereas none of the noncarcinogens tested

Table 6-29. HAMSTER EMBRYO CELL TRANSFORMATION PRODUCED BY SEVERAL POLYCYCLIC HYDROCARBONS AND THEIR DERIVATIVES

Compound	Concentration, µg/ml	Total number of colonies	Cloning efficiency, %	Number of transformed colonies	% transformation	Reference
DB[a,h]A[a]	2.5	760	4.2	4	0.5	301
	5	690	3.8	4	0.7	301
	10	790	4.4	7	0.9	301
DB[a,h]A[b]	2.5	1341	13.4	3	0.2	300
	5.0	1363	14.0	11	0.8	300
	10	1365	14.5	7	0.5	300
DB[a,h]A5,6-epoxide[a]	2.5	598	3.3	3	0.5	301
	5	601	3.3	12	2.0	301
	7.5	395	2.5	31	7.8	301
	10	350	1.9	14	4.0	301
DB[a,h]A5,6-epoxide[b]	2.5	895	10.1	7	0.8	300
	5.0	866	9.3	20	2.3	300
	7.5	817	9.3	22	2.7	300
	10	707	7.7	30	4.2	300
MCA[c]	2.5	404	10.1	9	2.2	301
	5	370	9.2	10	2.7	301
	7.5	349	8.7	15	4.3	301
MCA[d]	2.5	664	9.6	20	3.46	303
MCA epoxide[c]	3.5	364	2.4	13	3.6	301
	5	245	1.5	8	3.3	301
	7	103	0.7	17	16.5	301
BaP[d]	1	1016	8.46	25	2.46	303
	5	394	7.17	21	5.33	303

[a] 7-day treatment of cells seeded on a feeder layer.

[b] 7-8 day treatment of cells.

[c] 4-hour treatment of cells seeded in conditioned medium.

[d] 8-day treatment of cells.

showed any activity. Moreover, many of the carcinogens which have not been shown to be mutagenic toward S. typhimurium in vitro (e.g., chrysene) were capable of transforming the hamster cells. Calculations have been made which show that a battery of tests using S. typhimurium (Ames assay), polymerase A-deficient E. coli, and hamster embryo cell transformation are capable of detecting nearly all carcinogens tested, both POM and non-POM types.

It is known that alteration of microsomal enzyme activity either in vitro or in vivo can have a marked effect on the carcinogenic response to POM. Nesnow and Heidelberger[306] reported that in 10T1/2CL8 cells, a line of contact-sensitive C3H mouse embryo fibroblasts, transformation in culture was altered by chemical modifiers of microsomal enzymes. Pretreatment of 10T1/2C18 cells with benz[a]anthracene, a microsomal enzyme inducer, caused a doubling in MCA-mediated transformation. Similarly, treatment with inhibitors of epoxide hydrase (e.g., cyclohexene oxide; styrene oxide; 1,2,3,4-tetrahydronaphthalene-1,2-oxide) caused an increase in transformation over that obtained with MCA treatment alone. Thus, it seemed that treatments which can induce epoxide-forming enzymes and/or lower the activity of epoxide-degrading enzymes will enhance the degree of transformation in cultured cells by altering steady-state levels of oncogenic epoxides.

Chen and Heidelberger[307,308] developed a system (which is no longer used) using C3H mouse ventral prostate cells to examine transformation by carcinogenic hydrocarbons under conditions in which no spontaneous malignant transformation occurred. Cells treated with MCA (1 μg/ml) for 6 days in culture produced malignant fibrosarcomas in 100 percent of mice into which they were subcutaneously injected. When treated for only one day with MCA at the single cell stage, 100 percent of the clones were transformed to malignancy. A good quantitative correlation was obtained between the in vivo oncogenic activity of eight hydrocarbons (including BaP, MCA, DMBA, and DBahA) and the number of transformed colonies produced in this system. In contrast to the enhanced transforming ability of K-region epoxides relative to the parent hydrocarbon in hamster embryo cells, the K-region epoxide derived from DMBA was less active and the K-region epoxides from MCA, DBahA, and benz[a]anthracene were more active than the parent compound in mouse prostate cells.[309,310] Moreover, the epoxide derived from DMBA was more toxic than DMBA itself. The anomalous behavior of DMBA may have been due, however, to a decreased intracellular half-life of the epoxide because of its greater chemical reactivity.

Transformation of mouse, rat, and hamster embryo cells by organic extracts of airborne particulate matter has been reported.[311-313] Cells preinfected with RNA tumor viruses led to accelerated transformation, as well as transformation in cells not inducible by crude extracts alone. Rhim and coworkers[312] reported that the benzene extract had 100 to 1000 times the cell transformation activity attributable to the pure BaP contained in it. In addition, the methanol extract, although containing only 1/30 as much BaP, showed activity comparable to the benzene fraction. Thus, a diversity of carcinogenic material (POM or otherwise) is apparently contained in extracts of particulate pollutions, a conclusion which is supported by in vivo studies (see Section 6.4).

Attempts to transform human cells in culture with POM (e.g., BaP, MCA, DMBA) have generally met with failure.[314] However, Rhim and coworkers[315] reported that a human osteosarcoma clonal cell line could be further transformed in vitro with DMBA. Morphologic alterations and abnormal growth patterns became evident in cells treated with DMBA at 2.5 and 1.0 μg/ml in the fifth subculture 52 to 57 days after exposure. One of the altered cell lines obtained from the 1 μg/ml treatment was tumorigenic in nude mice by subcutaneous and intracerebral injection. Interpretation of the significance of these results is made difficult by the fact that an aneuploid sarcomatous cell line had to be employed in order to demonstrate successful transformation.

The use of organ cultures for the assessment of chemical carcinogenicity suffers from the lack of reliable biochemical and morphological parameters for measuring early neoplastic changes. Nevertheless, pioneering work in the application of organ culture to chemical carcinogenesis was performed by Lasnitzki.[316] Microgram quantities of MCA added to organ cultures of rat and mouse prostate fragments caused extensive hyperplasia and squamous metaplasia. However, these preneoplastic morphological effects are generally not associated with subsequent tumor development when carcinogen-treated pieces of tissue are implanted into host animals.[291] Limited success has been achieved with organ cultures of rat tracheas, which showed characteristic morphologic alterations when treated with DMBA, BaP, and MCA.[291] In addition, Crocker[317] has exposed respiratory epithelia from the hamster, rat, dog, and monkey to BaP at 7 to 15 μg/ml and observed occasional squamous metaplasia. More commonly, pleomorphic cells in a dysplastic epithelium were evident as a result of the treatment. Using this system, it was also possible to demonstrate a protective effect of vitamin A against BaP-induced abnormal differentiation. It has been suggested that rat tracheas maintained in organ culture may be a useful system for the predictive screening of potential carcinogens.[318]

A unique organ culture technique has recently been reported in which BaP (4 or 12 mg) was administered to pregnant mice (strain A and C57 Bl), and lung tissue of their 19 to 20-day-old embryos was subsequently explanted in culture.[319] A transplacental influence of BaP was manifested as a proliferative stimulus in embryonic lung tissue. Hyperplasia arising in the bronchial epithelium led to the development of adenomas in a large percentage of the explants.

6.4.9 Dose-Response Models in Carcinogenesis

Published in the early 1940's, Bryan and Shimkin's[320] paper is a good summary of the "state of the art" in the design and analysis of experiments with carcinogenic agents. The development of tumors at local sites of injection with varying doses of carcinogenic hydrocarbons (MCA, BaP, DBahA) into mice or rats was characterized in terms of: (1) latent periods between injection and appearance of tumor, and (2) incidence (percent) of tumor bearing animals.

Assuming that the logarithms of minimal effective doses for individual animals are normally distributed, an S-shaped cumulative normal incidence curve is expected as a function of log dose. Consequently, the inverse transformation to probits is expected to fit a straight line. The authors presented data which fit this well, at least when doses were not extremely high. Average latent periods, on the other hand, appeared as a linearly decreasing function of log dose until a flat minimum was reached at high doses of carcinogen.

Since departures from parallelism in bioassays defy interpretation in terms of simple relative potencies, the authors recommended comparison of doses and mean latent periods at the 50 percent level of incidence. Other specific problems in the design and analysis of experiments in carcinogenesis were discussed: species and animal variability, planning of sample sizes, duration of observation, and competing risks of death.

The principal focus of this paper was on demonstrating the usefulness of formal statistical methods. No concern was expressed about the low-dose validity of the incidence and latency models, and no attempt was made to extrapolate to human populations. In the context of the times when this article was written, however, it is quite understandable that the authors had to set their sights on more modest objectives.

In 1953, Heston and Schneiderman[321] referred briefly to the initiation-promotion theory but subsequently focused on the question of whether one or more somatic gene mutations are involved in carcinogenesis. Specific reference was made to earlier experiments by Charles and Luce-Clausen who produced lung papillomas in mice painted repeatedly with BaP; the square roots of numbers of tumors per mouse were found to fit a linear relationship with dose. If this had been the result of single short-term exposures to carcinogens, it would suggest a two-step recessive mutation mechanism. However, the confounding of a long-term dose schedule with latency made interpretation unclear. Heston and Schneiderman therefore designed a new experiment using 2-month-old strain A mice given a single injection of 0, 0.1, 0.2, 0.3, 0.4, or 0.5 mg DBahA and sacrificed six months later.

The average numbers of tumors per mouse in the latter experiment exhibited a good fit to a straight line dose response and a poor quadratic fit, thus providing greater support for a one-step dominant than for a two-step recessive gene mutation mechanism.

The mean number of tumors at zero dose exceeded the linearly extrapolated value. This discrepancy from linearity was attributed by the authors to a combination of natural carcinogenic processes active at zero dose and deactivation or other pharmacological factors operating to discount nominal doses to lower effective levels. The authors mentioned a "threshold" dose of 0.03 mg under the experimental circumstances. However, they quickly rejected this idea in favor of speculation that the dose-response curve may have convex curvature in the 0 to 0.1 mg range of DBahA, presumably because of variability among the thresholds of individual mice.

Mantel and coworkers[322] considered the consequences of assuming a linear rise in expected numbers of tumors elicited by injecting a carcinogen into a mouse at doses above some threshold level. The authors interpreted the slope of the line as indicative of the animal's sensitivity to the carcinogen, once the dose exceeds the threshold level. It was shown that, in a population of mice having variable dose thresholds and variable sensitivities, the average number of tumors per mouse remains a linear function of dose at levels above the maximum value of the mouse thresholds. It was shown that at doses lower than this the relationship is convex.

A weighted least squares fit to a replicate experiment with DBahA gives additional support to the linearity result reported previously by Heston and Schneiderman.[321] While the authors also demonstrated a linear range of dose response with intraperitoneal urethane, deceleration at very high doses occurs, presumably as the result of competition between, or coalescence of, multiple tumors.

More important from the standpoint of safety regulations, it was shown that extrapolation of the straight portion of the dose response for groups of mice could give a biased estimate of the average threshold--an artificially high one if mice sensitivities are positively associated with their thresholds, but artificially low in the more likely case of decreasing sensitivity with an increasing threshold. In the latter case, a linear extrapolation would provide a conservatively low estimate of the average threshold. However, one may criticize the use of an "average threshold" as a parameter for safety. It is not ideal for such a purpose, since it does not directly indicate the proportion of individuals at risk of tumorigenesis at extrapolated low dose levels.

More recently, in 1976, Yanysheva and Antomonov[323] emphasized the importance of, but really provided no principles for, choosing animal species and schedules of exposure which might be appropriate for extrapolating to permissible levels in man. They, like others, were forced to use highly informal judgments in choosing their experimental conditions.

From 16 to 40 random-bred white rats at each of several doses received intratracheal BaP at monthly intervals for up to 10 months. Total administered doses ranged from 0 to 25 mg. Presumably (though this is not entirely clear) each rat was observed throughout its entire life in order to determine the age at onset, the location, and the histological type of the first malignant or benign tumor. The authors do not say how they determined the age at onset, and it is difficult to imagine that they could be very accurate in that endeavor unless special attention were given to the problem.

The reported lifetime incidences of tumor-bearing rats vary from 9.5 percent (non-lung sites) in controls and 0 percent in those at low dose levels of benzpyrene, to over 80 percent reported after 25 mg. Responses appeared as a concave increasing function of untransformed doses or as a convex function of log doses.

The reported "time to occurrence" of first tumor appeared to be the average of onset times of first tumors for affected rats at a given dose level, but the authors were not explicit about this. Generally, these times decreased with increasing doses in the authors' experience.

By a crude empirical fit, the expected occurrence time of the first observed tumor in an animal was given as:

$$T = \frac{1.021}{d} + 16.79$$

where T = average first appearance time and d = total dose (mg). Extrapolating from this, the authors estimated that a dose of 0.02 mg would correspond to first tumors at an expected time of 67.9 months. Since this exceeds the lifespan limit of such rats, it became the authors' recommended dose. One fault with this approach is that, even when correctly indicated, the expected time of first tumor onset is merely an average; many tumors could first appear within the lifetimes of members of the species, even if the extrapolated average exceeded the lifespan limit. Another major difficulty is lack of clarity as to whether, or how, the experimental experience should be extrapolated to man.

Cornfield[324] provided a valuable review of approaches to the regulation of involuntary exposure to harmful substances. Particular attention was given to carcinogens in food. In addition, he examined a formal kinetic model which suggests the existence of a threshold, or near-threshold, in the dose-response curve. Cornfield recognized the governmental regulatory principle of balancing risk against benefits, but the subsequent discussion focused on problems relating to risks as expressed by lifetime cancer incidences at low doses.

A regulatory rule-of-thumb is cited[324] by which the no-observed-effect level (NOEL) is divided by 100 to set an acceptable daily intake (ADI) tolerance specification for humans. Another technique is to extrapolate downward from doses that elicit observable lifetime incidences so as to reach doses expected to produce arbitrarily low incidences of the order of 10^{-6} to 10^{-8}. The difficulty cited here is in the diversity of results, depending on the choice of models which perform comparably well in ranges of observable responses.

The theoretical case for low dose linearity of response (down to 0 response at 0 dose) is favored by: (1) its conservativeness compared with probit model extrapolation; (2) an approximate low dose linearity in the one-hit Poisson-like model of carcinogenesis; and (3) by results of all too few epidemiological studies of large groups of people exposed to low doses (references by the author are to H.A. Kahn, "National Cancer Institute Monograph No. 19," 1966, and J. Cornfield et al., "Proceedings of the First International Toxicology Congress," in press).

At least when using formal models, Cornfield argues against the practice of mingling informal conservatism with extrapolated incidence expectations, since this may overly weight the risks of harm against offsetting benefits. A similar argument also would apply to using pessimism or optimism factors when estimating benefits.

Cornfield presented a kinetic model which yields steady state equations with interesting implications for dose response curves. The steady state solutions indicate:

1. No toxic reaction in a host as long as the quantity (moles) of toxic substance is less than the quantity (moles) of host deactivator, provided that the host deactivation process is irreversible.

2. No threshold, but an approximately linear dose-response at low dose levels, provided that the host deactivation process is reversible.

3. That any irreversible component in a vertical chain of deactivation steps results in a threshold.

4. That any incremental step in a chain of reversible deactivation components lowers the linear low dose slope.

One assumption in the underlying kinetic model which may not prove to be correct is that the lifetime probability of a toxic effect is said to be proportional to the steady state quantity of activated carcinogen. Another complication, established clearly by Mantel et al.[322] and explicitly recognized by Cornfield, is that individual thresholds (or near-thresholds) do not preclude convexity in the dose-response for groups of animals because of variability in individual thresholds.

Finally, and perhaps most important, the formal results apply only to steady-state conditions. Even in irreversible deactivation processes, there may be extended periods during which activated carcinogens can constitute a risk of harmful effects prior to the host achieving a steady state. A zero risk achieved in the steady state would apply only after the host had survived an initial period at risk. It further seems realistic to suppose that repeated pulses of carcinogenic exposures throughout life would complicate the dynamics of the process. Thus, the steady-state solutions of this formulation may not be particularly useful in establishing new rules for acceptably low doses of harmful substances in the human population. Perhaps a far greater potential for this kind of model may be in stimulating new investigations of variability in low dose thresholds in very large groups of different species and strains of experimental animals, possibly leading to the identification of endogenous deactivation mechanisms for carcinogenic substances and, consequently, the raising of thresholds in those species having increased capacity for carcinogen deactivation.

Unfortunately, the application of animal-derived dose-response relationships to human situations is not likely to provide reliable estimates of cancer risk. Assuming that the incidence of carcinoma is proportional to steady state levels of activated carcinogen, the dynamics of this process would be dependent on the species employed and the target tissue examined. Wide variation in both species susceptibility and metabolite kinetics are known to exist, and would require the compilation of numerous data sets to provide meaningful results. For human risk assessment, reliance on epidemiologic data, coupled with the apparently conservative assumption of low dose linearity of response, presently appears to be the most practical approach.

6.4.10 Synergistic and Antagonistic Interactive Effects

It is well-known that the development of polycyclic hydrocarbon-induced tumors can be altered by: (1) components in the diet, (2) agents which affect the activity of certain enzymes, (3) other co-administered noncarcinogenic or weakly carcinogenic chemicals, or (4) the vehicle used to deliver a carcinogen to experimental animals. These influences can have an important effect on possible threshold levels for tumorigenic response, although the mechanism of such interactive effects is unknown. These factors further complicate the extrapolation of experimental animal data to human situations.

Early work on the inhibition of carcinogenesis was reviewed by Falk and coworkers.[325] In addition, they conducted a series of studies to evaluate the carcinogenic action of MCA, DBahA, and BaP in the presence of closely related POM. Simultaneous administration of DBahA or MCA (30 µg) with 15 times the molar equivalent of their respective dihydro- or hexahydro-reduction products by subcutaneous injection to male C57 black mice caused either a complete inhibition or dramatic reduction of the normal carcinogenic response. Administration of the dihydro- and hexahydro-derivatives at intervals extending from 14 days prior to and seven days subsequent to DBahA (60 or 135 µg) injection also caused significant inhibition of tumor development. The injection vehicle (tricaprylin or ethyl laurate) also affected the inhibiting response. Injections of the noncarcinogen phenanthrene together with DBahA in molar ratios of 1:24 and 1:48 significantly reduced tumor yield. In simulated environmental studies BaP was administered to mice together with various noncarcinogenic POM commonly found in polluted atmospheres. The results (Figure 6-16) demonstrated that in all cases a marked inhibition of carcinogenesis resulted. Neutral fractions isolated from polluted urban atmospheres produced similar inhibitory effects with BaP.

The experiments of Falk and coworkers[325] were modified and repeated by Pfeiffer who obtained different results (see also Section 6.4.7). Ten noncarcinogenic polycyclic aromatic hydrocarbons found in automobile exhaust were tested in combination with BaP (3-100 µg) and DBahA (2-75 µg) by subcutaneous injection in 3000 female NMRI mice. The tumor incidence resulting from all 12 compounds being administered together could be attributed to the

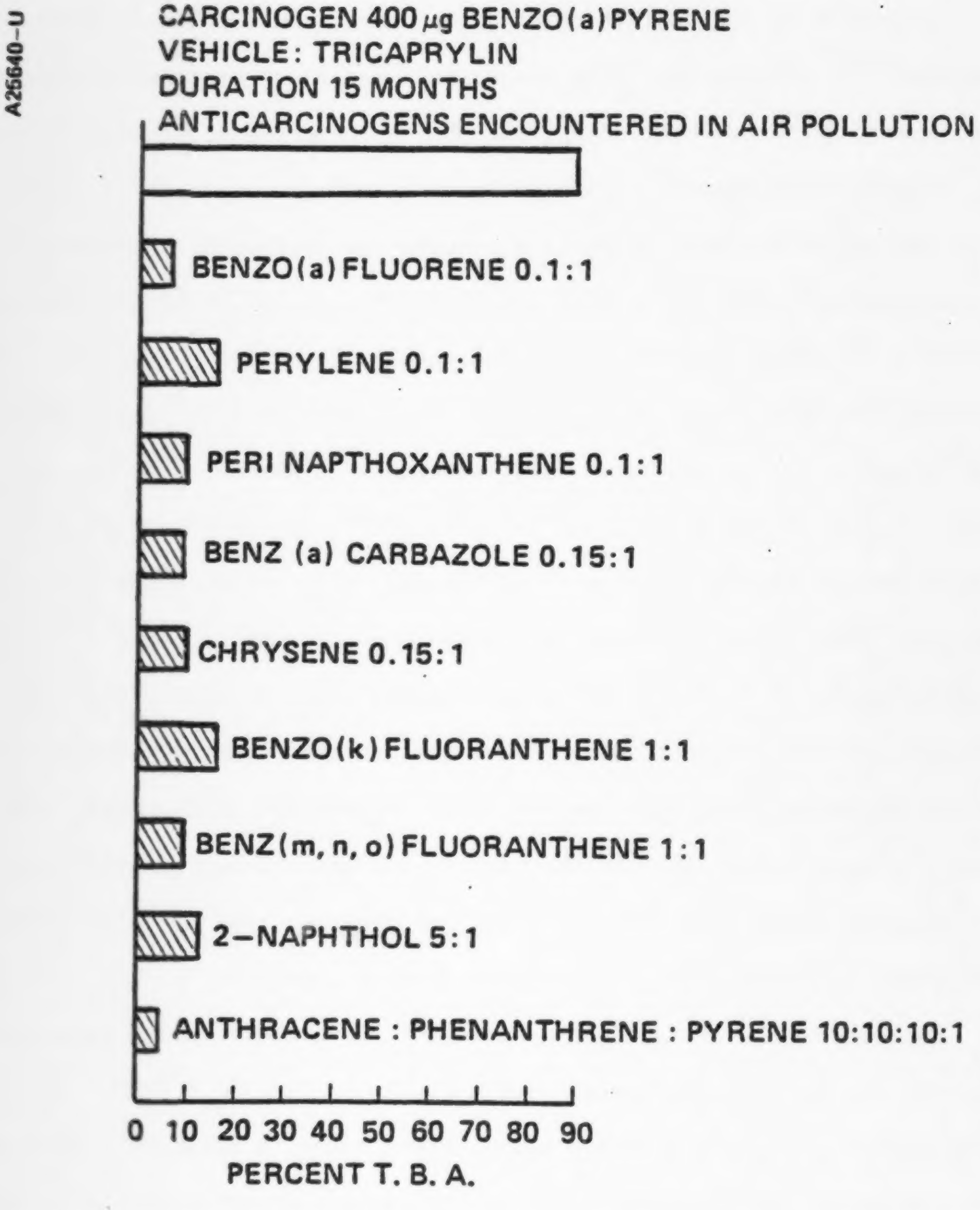

Figure 6-16. The inhibiting effect of hydrocarbons found in polluted urban air and cigarette smoke in concentrations approximating those occurring naturally when injected in combination with BaP.[325]

presence of DBahA, with little influence from BaP or the other ten chemicals. No inhibitory effect of the ten noncarcinogens was evident; moreover, an increased tumor yield resulted from injection of mixtures containing increasing amounts of the components. This effect, however, was less dramatic than if BaP were administered alone, and paralleled the dose-response curve of DBahA acting singly. It is not known how the relative tumor susceptibility of NMRI mice, as compared to C57 mice used by Falk, may have affected the results.

Many studies on cocarcinogenesis have been concerned with the identification of tumor-accelerating substances present in cigarette smoke. These compounds are generally tested for cocarcinogenic activity by repeated application to mouse skin together with low doses of BaP. A positive response would be obtained in cases where the tumor yield of the combination exceeds that produced by either agent alone at the same doses. Van Duuren and coworkers[206,326,327] established that a pronounced cocarcinogenic effect could be obtained with catechol and the noncarcinogens pyrene, BeP, and benzo[g,h,i]perylene. Doses of 2000, 12, 15, and 21 μg, respectively, were applied 3 times a week for 52 weeks to female ICR/Ha Swiss mice. Each animal also received 5 μg of BaP in 0.1 ml acetone with each dose of test substance. Although phenol has been regarded as a tumor-promoter in the two-stage carcinogenesis system, this compound had a slight inhibitory effect on BaP carcinogenesis when administered in combination. These results therefore indicated that tumor-promoters and cocarcinogens may not have the same mode of action, and that the two terms should not be used interchangeably. Other POM's (e.g., fluoranthene, pyrene, pyrogallol) also possess cocarcinogenic activity but have no tumor-promoting activity. Additional studies by Schmeltz and coworkers[328] established that most of the naphthalenes found in cigarette smoke have an inhibitory effect on skin tumorigenesis (250 μg, 3 times a week) as induced by BaP (3 μg, 3 times a week). On the other hand, several of the alkylnaphthalenes tested (dimethyl-, trimethyl-, tetramethyl-) enhanced the carcinogenic activity of BaP on mouse skin.

Numerous investigators have shown that antioxidants are effective inhibitors of POM-induced tumor development. This action has been demonstrated with selenium,[329-331] dl-α-tocopherol (Vitamin E),[329,330] and ascorbic acid[332] in mice treated with DMBA and croton oil. The carcinogenic action of MCA has been reduced by tocopherol-rich diets in rats and mice.[333,334] The antioxidant food additives butylated hydroxytoluene (BHT), ethoxyquin, and butylated hydroxyanisole (BHA) have inhibited lung, breast, and gastric tumor formation induced in rats and mice by various carcinogens in the diet.[335-337] The sulfur-containing antioxidants disulfuram, dimethyldithiocarbamate, and benzyl thiocyanate inhibited DMBA-induced mammary cancer in rats when they were added to the diet; in the mouse, disulfuram prevented the formation of forestomach tumors induced by BaP in the diet, but had no effect on BaP-induced pulmonary adenoma.[338] The agricultural herbicide, maleic hydrazide, and its precursor, maleic anhydride, can inhibit the initiating activity of DMBA in the mouse skin two-stage carcinogenesis system.[339]

Investigators have attempted to explain the mechanism for the anticarcinogenic action of antioxidant chemicals. Chromosomal breakage in human leukocyte cultures induced by DMBA was reduced by simultaneous addition of ascorbic acid (31.7 percent reduction), butylated hydroxytoluene

(63.8 percent), Na_2SeO_3 (42.0 percent), or dl-α-tocopherol (63.2 percent).[340,341] It was not apparent from this study whether anticarcinogenesis by antioxidants may be due to a direct protective effect against chromosome damage or to an interference with metabolic activation of carcinogens, although the latter seems most likely. This aspect was further pursued by Rahimtula and coworkers[342] who examined the abilities of several antioxidants to affect BaP hydroxylation by rat liver microsomal mixed-function oxidases. Their results indicated that antioxidants can markedly inhibit BaP hydroxylation by an apparently direct action on microsomal oxidation mechanisms. Furthermore, all of the antioxidants tested reduced the bacterial mutagenicity of BaP in the presence of rat liver microsomes and cofactors (Table 6-30). The authors suggested that antioxidants may exert their protective effect in vivo by inhibiting the formation of carcinogenic intermediates from POM. This conclusion, however, seems to conflict with data indicating that inducers of increased BaP hydroxylase activity can also inhibit tumor formation.[343] However, flavones are also inhibitors of BaP metabolism in vitro, thereby indicating that their specific effects depend upon how and where they are used. These investigators found that several synthetic and naturally occurring flavones, when incorporated in the diet (3 to 5 mg/g) or applied to the skin, caused a profound increase in BaP hydroxylase activity in the small intestine and skin, respectively. In addition, pulmonary adenoma formation resulting from oral administration of BaP was totally prevented and skin tumors initiated by BaP application to mice were significantly reduced (>50 percent) by treatment with the synthetic flavone, β-naphthoflavone. Pulmonary tumor formation was also reduced 50 percent by incorporation of the naturally occurring flavone,

Table 6-30. EFFECT OF ANTIOXIDANTS ON BENZO[a]PYRENE METABOLISM AS ASSAYED BY *Salmonella typhimurium* (STRAIN TA98) MUTAGENICITY IN THE PRESENCE OF RAT LIVER MICROSOMAL FRACTION AND NADPH[342]

Antioxidant	Concentration	Revertants of strain TA 98 per plate
None		384
Butylated hydroxyanisole	100 μM	193
Ethoxyquin	25 μM	148
Pryogallol	100 μM	156
Glutathione	1 mM	92

quercetin pentamethyl ether, into the diet. The possibility that only specific components of the drug-metabolizing enzyme system may be induced by antioxidants has not been fully explored. Sullivan and coworkers[344] recently demonstrated that BHA, BHT, phenothiazine, phenothiazine methosulfate, and ethoxyquin can all reduce the quantitative yield of BaP metabolites in incubations with rat liver microsomes. On the other hand, these antioxidants did not react with 6-oxy-BaP free radical, although some reduction in the enzymatic in vitro formation of 6-oxy-BaP free radical could be obtained. The authors concluded that the antioxidant effect on BaP tumorigenesis is probably not due to a direct reaction with BaP free radicals.

Benson and coworkers[345] recently provided evidence suggesting that the protective effects of antioxidants are mediated, at least in part, by an elevation of glutathione S-transferase activity. The incorporation of BHA or ethoxyquin in the diet of mice greatly reduced the conversion of BaP to mutagenic metabolites that were subsequently excreted in the urine. In both rats and mice, BHA in the diet increased hepatic glutathione S-transferase specific activities. The mutagenic activity of BaP metabolites in the urine could be reduced by the addition of glutathione together with either purified glutathione S-transferases or liver cytosols from rats or mice fed on BHA-containing diets. The authors cited several studies indicating that various epoxide metabolites of POM are substrates for conjugation with glutathione. These studies are considered as important supporting evidence for the role of conjugation with glutathione in the detoxification of carcinogenic POM.

In addition to flavones, other naturally occurring compounds have exhibited protective effects against POM-induced tumor formation. Vitamin A has clearly been shown to play a role in reducing carcinogen-induced tumors.[346-349] Nettesheim and Williams[350] recently undertook to determine whether inadequate vitamin A consumption may predispose individuals to carcinogenesis, or whether increased vitamin A intake exerts a protective effect against neoplasia. They found that a diet deficient in vitamin A increased the formation of MCA-induced metaplastic lung nodules in female Fisher 344 rats, even though adequate amounts of the vitamin were stored in the liver. On the other hand, moderate amounts of vitamin A added to the diet markedly reduced the development of MCA-induced lesions of the lung. High doses of the vitamin given intragastrically provided no additional protection, however.

Further studies on naturally occurring antineoplastic compounds were recently reported by Wattenberg.[351] Benzyl isothiocyanate and phenethyl isothiocyanate, both found in cruciferous plants such as cabbage, brussel sprouts, cauliflower, etc., inhibited DMBA-induced mammary cancer in Sprague-Dawley rats. When added to the diet together with DMBA, these compounds inhibited the development of forestomach tumors and pulmonary adenomas in female ICR/Ha mice. Similar anticarcinogenic actions were obtained when BaP was incorporated into the diet. These results lead to interesting speculation regarding the role and importance of diet in human susceptibility to environmental carcinogens. In cases where dietary constituents can alter the metabolism of xenobiotics such as POM's, the anticarcinogenic effect may result from an alteration of steady state levels of activated versus detoxified metabolites.

Studies have shown that not only can specific substances in the diet affect the response to carcinogens, but protein deprivation in general may also decrease the activation of carcinogens.[352] The feeding of protein-deficient diets to male mice caused decreased liver weights and reduced cytochrome P-450 content in the total liver. Diets deficient in both protein and choline produced even further reductions in liver weight and cytochrome P-450 content. Liver microsomes isolated from these animals displayed a decreased ability to activate dimethylnitrosamine to a mutagen (in the Ames *Salmonella* test system), which paralleled the reduction in cytochrome P-450 content produced by the diet. Conversely, the inactivation of the direct-acting (ultimate) carcinogen N-methyl-N'-nitro-N-nitrosoguanidine was reduced in liver microsomes from mice receiving a protein-deficient diet.

6.5 MUTAGENESIS

It is presently believed that fundamental similarities exist between the molecular mechanisms of mutagenesis and carcinogenesis induced by POM. These assumptions are based on the theory that highly electrophilic metabolites (e.g., epoxides) are generated from POM by the action of the MFO system (see Section 6.2). These reactive intermediates are capable of binding to DNA and/or other critical cellular macromolecules (see Section 6.2.6) presumably to initiate a mutagenic or carcinogenic transformation.[353] The concept that carcinogenesis is an expression of an alteration in the genetic material of a treated cell (i.e., somatic mutation) implies that a relationship exists between mutagenesis and carcinogenesis.[133] Therefore, it is believed that an investigation of the mutagenicity of POM: (1) may be predictive of carcinogenic potential, (2) may help elucidate the mechanism for malignant transformation in certain systems, and (3) may indicate the presence of a potential threat to human health posed by a particular POM.

6.5.1 *In Vitro* Studies

6.5.1.1 *Microbial systems*--Early attempts to demonstrate mutagenicity by POM failed because of the inability of microorganisms to metabolize these compounds to reactive derivatives. The addition of subcellular preparations and the development of sensitive tester strains of *Salmonella typhimurium* have resulted in a mutagenesis test system (Ames assay) now receiving wide application as a powerful screening tool for evaluating environmental agents.[354] All of these strains are histidine-dependent and can be reverted to histidine independence by mutation. Mutagenicity in the Salmonella/microsome system displays a high correlation with carcinogenicity for many chemicals, although exceptions are known to occur with POM (see Section 6.5.3). Teranishi and coworkers[355] found that a quantitative correlation existed between carcinogenicity and mutagenicity of seven polycyclic hydrocarbons when strain TA1538 was tested using rat liver enzymes. In discussing the significance of the Salmonella/microsome assay, McCann and Ames[356] believed that their results supported the somatic mutation theory of cancer, and that a common link between mutagenesis and carcinogenesis by chemicals is the ability to produce DNA damage.

In Japan, a data base has been developed on 60 chemicals (POM and non-POM) which correlates mutagenic activity in various strains of *S. typhimurium in vitro* with carcinogenicity *in vivo*.[357] With the use of an activating system from liver microsomes and the introduction of the R-factor plasmid into *S. typhimurium* for increased sensitivity, a strong correlation between mutagenicity and carcinogenicity was obtained. Studies conducted in the United States on 102 compounds tested *in vitro* using *S. typhimurium* generally confirm the high capability of this system to detect carcinogens.[358] In addition, a further improvement in the detection of carcinogens could be obtained by combining the results from *Salmonella* with those from polymerase A-deficient *E. coli*. On the other hand, the use of *S. typhimurium in vivo* (host-mediated assay) was considerably less reliable for the detection of carcinogenic hydrocarbons and aromatic amines.[358]

In recent studies, the Ames *Salmonella* test system has been employed to assess the mutagenicity of organic extracts of airborne particulate pollutants.[359] Although the total extract displayed marked mutagenicity, assays of various subfractions of the extract indicated that much of the activity is due to compounds other than polycyclic hydrocarbons and do not require metabolic activation. These results, which are supported by data from in vivo studies (see Section 6.4), indicate that caution must be exercised in attributing the mutagenic/carcinogenic effects of air pollutants solely to the presence of polycyclic hydrocarbons. The recent demonstration of the mutagenicity of quinoline and its 5-hydroxy- and 8-hydroxy-derivatives in the Ames assay emphasizes the potential role of nitrogen-containing POM's as hazardous air pollutants.[360]

Several years ago a joint program was undertaken by scientists at the National Institute of Arthritis, Metabolism, and Digestive Diseases, and at the Department of Biochemistry and Drug Metabolism of Hoffmann-LaRoche, Inc., to determine the structures of POM metabolites that are responsible for their mutagenicity and carcinogenicity. It had been previously demonstrated that epoxides of carcinogenic POM are frameshift mutagens.[361] In 1975, it was established that the K-region BaP 4,5-oxide[362] and non-K-region BaP 7,8-diol[363] (with metabolic activation) were more mutagenic than the parent compound in *S. typhimurium* strains TA100 and TA1538. Subsequently, nearly all of the possible primary oxidative metabolites of BaP were synthesized and tested for mutagenicity.[364-368] The results of these tests have been recently reviewed[48,64,65] and are summarized in Table 6-31. It was emphasized that diol epoxide-1 ((±)-7β,8α-dihydroxy-9β,10-βepoxy-7,8,9,10-tetrahydro BaP) is a very potent mutagen in *S. typhimurium*, and that the BaP 7,8-diol-9,10-epoxides are likely candidates as the ultimate biologically reactive metabolites of BaP.[367] It was subsequently demonstrated by these researchers that the mutagenicity of

Table 6-31. COMPARISON OF INHERENT MUTAGENIC ACTIVITY OF THIRTY BaP DERIVATIVES IN *Salmonella typhimurium* TA98 AND IN CHINESE HAMSTER V79 CELLS[a] 64

Compound[b]	Relative % activity	
	Strain TA98	V79
Diol epoxide-1	100	40
Diol epoxide-2	35	100
H_4 9,10-epoxide	95	40
H_4 7,8-epoxide	10	0.2
BaP 4,5-oxide	20	1
BaP 7,8-oxide	1	<0.1
BaP 9,10-oxide	1	<0.1
BaP 11,12-oxide	0.5	1
6-HOBaP	5	0.3
12-HOBaP	1.5	<0.1
1-HOBaP	0.5	0.1
3-HOBaP	0.5	<0.1
2-, 4-, 5-, 7-, 8-, 9-, 10-, 11-HOBaP	<0.1	<0.1
BaP 1,6-, 3,6-, 6,12-, 4,5-, 11,12-quinone	<0.1	<0.1
BaP 4,5-, 7,8-, 9,10-, 11,12-dihydrodiol	<0.1	<0.1
BaP	<0.1	<0.1

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[a] The relative percent mutagenic activities are approximations since the data were compiled from several separate studies conducted at different times. In some experiments, BaP 7,8-dihydrodiol was 0.1-0.4% as active as diol epoxide-2 in V79 cells.

[b] Abbreviations used: BaP, benzo[a]pyrene; 1-HOBaP, 1-hydroxybenzo[a]pyrene; 2- to 12-HOBaP, other BaP phenols; BaP 1,6-quinone, benzo[a]pyrene 1,6-quinone; BaP 3,6-quinone, BaP 4,5-quinone, BaP 6,12-quinone, and BaP 11, 12-quinone, other BaP quinones; BaP 4,5-dihydrodiol, *trans*-4,5-dihydroxy-4,5-dihydrobenzo[a]pyrene; BaP 7,8-, 9,10- and 11, 12-dihydrodiol, other dihydrodiols of BaP; BaP 4,5-oxide, benzo[a]pyrene 4,5-oxide; BaP 7,8-, 9,10- and 11,12-oxide, other BaP oxides; diol epoxide-1 (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro BaP; diol epoxide-2, (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro BaP; H_4 9,10-epoxide, 9,10-epoxy-7,8,9,10-tetrahydro BaP; H_4 7,8-epoxide, 7,9-epoxy-7,8,9,10-tetrahydro BaP.

the optical enantiomers of the diastereomeric BaP 7,8-diol-9,10-epoxides is highly stereospecific,[72] but does not correlate with carcinogenic activity for these same isomers. These results are of considerable significance in light of evidence indicating that the *in vitro* metabolism of BaP is stereoselective, depending upon the source of the microsomes and conditions of the incubation,[47] and suggests that the Ames assay may not be capable of distinguishing between certain stereoisomers. On the other hand, certain mammalian cells in culture (e.g., hamster V79 cells) are capable of detecting the stereoisomer of BaP diol-epoxide which is active *in vivo*.

Further evidence implicating the obligatory role of epoxide intermediates in the mutagenicity of BaP was reported by Oesch and coworkers.[369] They stated in support of this contention that: (1) epoxide hydrase inhibitors potentiated BaP mutagenicity, even though epoxide hydrase activity is necessary for the conversion of BaP-oxides to BaP-dihydrodiols prior to formation of the diol-epoxide; and (2) homogeneous epoxide hydrase prevented mutagenicity even though it is required for the formation of diol epoxides from BaP-oxides.

In this same regard, Levin and coworkers[366,370] reported on extensive studies into the role of epoxide hydrase in the metabolic activation of BaP to mutagenic products. The addition of purified epoxide hydrase decreased mutation frequency in *S. typhimurium* by 30 to 50 percent. However, mutagenicity could not be completely abolished by high levels of epoxide hydrase. Evidence showing that mutations induced by BaP 4,5-oxide could be reduced 90 percent by the addition of very small quantities (units) of epoxide hydrase argued against a central role for the K-region epoxide. Furthermore, these researchers established that epoxide hydrase converts BaP 7,8-oxide to BaP 7,8-dihydrodiol, which is further metabolized by the monoxygenase system to the highly mutagenic BaP diol epoxides. Moreover, these proposed ultimate mutagens were not substrates for epoxide hydrase, and therefore were not affected by addition of the purified enyzme.

A marked effect on BaP mutagenicity in the Ames assay has been achieved by the addition of a purified rat liver dihydrodiol dehydrogenase enzyme to the incubation system.[371] When BaP was activated by liver microsomes from MCA-pretreated rats, the addition of dihydrodiol dehydrogenase caused up to a 60 percent reduction in mutagenicity. From these data it was concluded that diol epoxide metabolites play a significant role in BaP-induced mutagenesis, and that the discovery of the action of dihydrodiol dehydrogenase in reducing this effect may have important implications for the control of metabolic pathways involved in carcinogenic bioactivation. The fact that dihydrodiol dehydrogenase did not completely abolish mutagenic activity indicated that BaP metabolites other than diol epoxides may also be mutagenic. This possibility is supported by the observation that certain simple epoxides, phenols, and phenol derivatives of BaP are mutagenic in the Ames assay.[372]

Support for the theory that diol epoxide metabolites of POM are ultimate mutagens in the Ames assay has been obtained from studies with MCA, DMBA, BA, 7-methylbenz[a]anthracene, 5-methylchrysene, chrysene, and DB(ah)A.[373,374,535,539] In particular, it was shown that the immediate dihydrodiol precursor of the bay-region (see Section 6.4) diol epoxide for each of these compounds was consistently the most highly mutagenic derivative.

6.5.1.2 Somatic cells in culture--Current methods of identifying chemically induced mutants from cultured mammalian cells rely upon the mass selection of variants resistant to the cytotoxicity of certain drugs (e.g., 8-azaguanine, 6-thioguanine, ouabain). Among the most suitable cells for this type of study are Chinese hamster cell lines, particularly V79 cells derived from male lung tissue. However, V79 cells apparently do not have the capacity for metabolic activation of POM.[375]

In 1974, Huberman and Sachs[376] reported a successful system for cell-mediated mutagenesis which combines V79 cells for the identification of mutants and lethally irradiated rodent embryo "feeder" cells for the metabolic activation of test chemicals. Mutagenicity was obtained with DMBA, MCA, and BaP at rates which correlated with their respective carcinogenic potencies. Large numbers of 8-azaguanine-resistant mutants were produced by treatment with as little as 0.1 μg/ml (Table 6-32). The noncarcinogen benz[a]anthracene produced no mutations in this system.

Huberman and Sachs[377] subsequently conducted expanded studies on the mutagenicity of ten polycyclic hydrocarbons at three different genetic loci in hamster cells. V79 cells (together with metabolizing cells) were used for identification of ouabain- and 8-azaguanine-resistant mutants induced by these polycyclics. The induction of temperature-resistant mutants was also examined by using Chinese hamster ovary cells derived from a clone of temperature-sensitive cells. Tables 6-33 and 6-34 summarize their results and indicate that DMBA was consistently the most potent mutagen tested, having significant activity at concentrations as low as 0.01 μg/ml.

The V79 cell system without the addition of metabolizing cells has been used to identify structures which may be the ultimate mutagenic/carcinogenic

Table 6-32. MUTAGENICITY OF CARCINOGENIC HYDROCARBONS TO CHINESE HAMSTER V79 CELLS CO-CULTIVATED WITH SYRIAN GOLDEN HAMSTER EMBRYO CELLS[a] 376

Treatment	Concentration of carcinogen, μg/ml	Number of V79 cells 2 days after treatment, $\times 10^{-6}$	Cloning efficiency of the V79 cells, %	Number of 8-azaguanine-resistant mutants/10^5 survivors
Solvent	0	3.0	99	7
7,12-Dimethylbenz[a]anthracene	0.1	1.3	7	270
3-Methylcholanthrene	0.1	2.3	78	110
Benzo[a]pyrene	0.1	2.7	89	70
Benzo[a]pyrene	1.0	1.9	15	320

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[a] The cultures were treated for 2 days with carcinogenic hydrocarbons. The cells were then trypsinized and seeded to determine the cloning efficiency of the V79 cells and the frequency of 8-azaguanine-resistant mutants; co-cultivation was with 2 x 10-6 irradiated golden hamster cells per Petri dish.

Table 6-33. INDUCTION OF OUABAIN- AND 8-AZAGUANINE-RESISTANT MUTANTS BY DIFFERENT CHEMICAL CARCINOGENS[377]

Treatment[a]	Cloning efficiency, %	Ouabain-resistant mutants per 10^6 survivors	8-Azaguanine-resistant mutants per 10^5 survivors
Solvent	92*	1	6
Pyrene	94*	1	5
Phenanthrene	79*	1	8
Chrysene	85*	2	9
Benz[a]anthracene	92*	2	9
Dibenz[a,c]anthracene	95*	3	22
Dibenz[a,h]anthracene	79*	4	17
7-Methylbenz[a]anthracene	61*	24	75
3-Methylcholanthrene	49**	14	72
	41†	38	152
	38*	108	366
	47**	18	54
Benzo[a]pyrene	27†	45	128
	21*	121	425
7,12-Dimethylbenz[a]anthracene	50§	22	41
	2**	66	281

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[a] The cells were treated for 2 days with the polycyclic hydrocarbons with *1 μg/ml, †0.3 μg/ml, **0.1 μg/ml, or §0.01 μg/ml. After treatment there were with 0.01-0.1 μg/ml of DMBA, 0.3-1 μg/ml of BaP, and 0.3-1 μg/ml of MCA, 1.0 to 2.5 x 10^6 V79 cells per petri dish. In all other cases, there were 2.7 to 3.6 x 10^6 V79 cells per petri dish.

Table 6-34. INDUCTION OF TEMPERATURE-RESISTANT MUTANTS IN CHINESE HAMSTER OVARY TEMPERATURE-SENSITIVE CELLS BY DIFFERENT CHEMICAL CARCINOGENS[377]

Treatment[a]	Concentration, μg/ml	Cloning efficiency, %	Temperature-resistant mutants per 10^6 survivors
Solvent	0	93	0.6
Pyrene	1.0	88	0.9
Phenanthrene	1.0	88	0.7
Benz[a]anthracene	1.0	78	0.7
Benz[a]pyrene	0.1	67	21
	0.3	45	56
	1.0	9	170
3-Methylcholanthrene	0.1	78	20
	0.3	32	46
	1.0	10	125
7,12-Dimethylbenz[a]anthracene	0.01	65	51
	0.1	3	494

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[a] Three days after treatment with the polycyclic hydrocarbons at 34°, the cells were seeded to determine the cloning efficiency of the ts cells and the frequency of temperature-resistant mutants. For selection of temperature-resistant mutants, the ts cells were shifted from 34° to 39°, 3 days after cell seeding. At the time when the cells were seeded for cloning efficiency and the frequency of mutations, there were after treatment with 0.01-0.1 μg/ml of DMBA, 0.3-1 μg/ml of BaP or MCA, 0.5 to 1.9 x 10^6 ts cells per petri dish. In all other cases, there were 2.1 to 2.3 x 10^6 ts cells per petri dish.

metabolites of BaP.[64,65,72,364,367,378] Numerous oxidative metabolites of BaP were surveyed, and the stereoisomeric BaP 7,8-diol-9,10-epoxides were found to be highly mutagenic (Table 6-31). The very short half-life (30 seconds) for BaP 7β,8α-diol-9β,10β-epoxide in tissue culture medium made comparisons of activity between the two diol epoxides very difficult, however. Nevertheless, the high mutagenicity of both diol epoxides, coupled with the high carcinogenicity of their precursors, BaP 7,8-dihydrodiol and BaP 7,8-oxide, suggests a strong formal relationship between mutagenicity and carcinogenicity insofar as a common pathway of metabolic activation is concerned.

Modifications of the V79 cell-mediated mutagenesis system have recently been developed which involve new means of metabolizing test compounds. Newbold and coworkers[379] combined baby Syrian hamster kidney cells (BHK21) for metabolism of POM with V79 cells for detection of ouabain- and 8-azaguanine-resistant mutants. This system produced binding reactions between DNA and BaP or 7-methylbenz[a]anthracene which accompanied mutagenesis in the same cells and were typical of those which occur *in vivo* or in primary cell cultures. Chromatographic analyses of the BaP-DNA reaction indicated that BaP 7,8-diol-9,10-epoxide was involved in the binding interaction. These results strengthened the argument which supports the role of diol epoxides in BaP-mediated mutagenesis and carcinogenesis. Furthermore, the cell-mediated mutagenesis system as modified and validated by Newbold et al.[379] is proposed as a convenient and representative mammalian cell screening system which may prove to be superior to mutagenicity assays with *S. typhimurium* in providing a rank-order correlation with carcinogenic potency in mammals (e.g., Iball index). However, additional screening of polycyclic hydrocarbons and aza-arenes is required before a definitive conclusion may be drawn for these classes of carcinogens.

The current belief that neoplastic transformation may arise from a chemically induced somatic mutation was made more convincing by the recent studies of Huberman and coworkers.[380] They demonstrated for the first time that BaP and BaP 7,8-dihydrodiol can induce both neoplastic transformation and mutagenesis (ouabain resistance) in the same normal diploid hamster embryo cells. A ratio between transformation and mutagenesis for ouabain resistance was only about 20:1.

A further adaptation of the V79 cell-mediated mutagenesis system has been employed which provides metabolic activation by rat liver homogenates containing microsomes and cofactors.[373,381] Mutants of V79 cells were detected by their resistance to the cytotoxic effects of 6-thioguanine. The mutagenic activity of BaP, MCA, DMBA, and benz[a]anthracene in this system showed a limited correlation with their respective carcinogenic potencies. With DBahA and DBacA, an inverse relationship between mutagenicity and carcinogenicity was obtained. Such a relationship has also been shown for the dibenzanthracenes when tested in the Salmonella/microsome assay.[355] Although mammalian cell-mediated mutagenesis is hindered because it is technically more difficult, time-consuming, and thus permits fewer compounds to be screened, there are advantages over the microbial system: (1) bacteria indicate reverse mutations and V79 cells indicate forward mutations, the latter type of mutation being more relevant to the process of neoplastic transformation; and (2) cells can be employed which are the same kind as found in the actual targets for mutagenesis and carcinogenesis induced by chemicals.

The analysis of chromosomal aberrations and sister chromatid exchanges (SCE's) is often recommended as a screening technique for potential mutagens and carcinogens. Several investigators have examined the effects of POM on the chromosomes of mammalian cells. Early studies indicated that variations in chromosome number and structure may accompany tumors induced by BaP, MCA, and DMBA in the rat, mouse, and hamster.[382] However, in cultured human leukocytes exposed to DMBA, chromosome damage was not the same as that produced in hamster cells. Although it is argued that chromosome changes in POM-induced tumors are nonrandom,[382] others[141,385] claim that detectable chromosome changes are not specific for the carcinogenic agent nor are they a prerequisite for neoplastic growth. Moreover, it was shown that an increased rate of SCE's could be produced by BaP in cultured human lymphocytes[386,387] but this was not correlated with different rates of BaP metabolism;[387] a surprising result in light of the known importance of metabolic activation for BaP mutagenicity. BaP-induced SCE's rates did not differ between lymphocytes taken from normal humans and those from patients with lung cancer.[387] In recent studies with cultured Chinese hamster cells exposed to DMBA, BaP, and MCA, none of the chemicals produced chromosome breaks and only DMBA could successfully induce SCE's.[388] Although it cannot be denied that POM causes chromosome damage, it is not clear whether this effect may represent an epigenetic phenomenon which is merely secondary to mutagenesis and neoplastic transformation. Furthermore, in cases where a chemically-induced mutation is "silent" (i.e., neutral amino acid substitutions), there is no reason to believe that detectable chromosome damage should occur.

In recent comparisons of three cytogenetic tests, (1) induction of chromosome aberrations, (2) induction of micronuclei, and (3) *in vivo* induction of sister chromatid exchanges, the latter test proved to be the most sensitive with carcinogenic polycyclic hydrocarbons.[389] However, positive results were also obtained with phenanthrene and thus limits the usefulness of sister chromatid exchange as a screening technique for carcinogen detection. BaP was positive in the sister chromatid exchange test, weakly active in the chromosome aberration test, and negative in the micronucleus test. On the other hand, DMBA was clearly positive in all three tests. It was concluded that cytological tests do not provide reliable correlations with all carcinogens tested and thus cannot be used alone in mutagenicity/carcinogenicity evaluations.

Damage to the genome resulting from chemical insult can theoretically also be detected by examining DNA repair.[390] The suggestion that DNA repair is applicable as a screening procedure for evaluating potential chemical mutagens is based on the assumption that the level of DNA repair synthesis in a cell reflects the extent of DNA damage produced by a chemical. Indeed, unscheduled incorporation of ^{3}H-thymidine into nuclear DNA of normal human cells exposed to epoxides of benz[a]anthracene and MCA has been observed.[390] However, since a metabolic activation system was not present in this system, the parent hydrocarbons showed no activity. More recent studies confirmed that K-region epoxides of BaP, DMBA, and DBahA caused DNA damage in human skin fibroblasts which was repaired with the same system used for repairing lesions induced by ultraviolet radiation.[391] As would be expected, the parent hydrocarbons exerted no effect. More importantly, results were obtained which indicated that the DNA repair process itself does not induce mutations, but rather that mutagenesis occurs before the DNA lesion can be excised. In fact, there is no *a priori* reason why POM should exert a mutagenic effect by inducing DNA lesions which require any kind of repair synthesis at all.

Nevertheless, DNA repair synthesis in human fibroblasts,[392-394] rat liver cells,[395] and Chinese hamster V79 cells[396] has been successfully used for the detection of chemical carcinogens, including numerous POM. However, the percentage of carcinogens giving positive results for DNA repair is considerably less than in the cell transformation or microbial mutagenesis assays. For example, benz[a]anthracene is negative in the DNA repair assay utilizing human or rat liver cells, and BaP is only positive in the DNA damage assay utilizing hamster V79 cells.

6.5.2 In Vivo Studies

6.5.2.1 Effects in somatic tissues--Tumors induced *in vivo* by POM are commonly associated with chromosome abnormalities in the neoplastic cells. In particular, sarcomas induced by DMBA, MCA, and BaP in the rat display karyotype variations which were reportedly nonrandom and distinctly different from sarcomas induced by Rous sarcoma virus.[383,397] The chromosome patterns of DMBA-induced sarcomas were found to be identical with those observed in primary rat leukemias[399] and primary carcinomas of the auricular skin[384] induced by DMBA. Consistent chromosome abnormalities in DMBA-induced rat leukemias were first reported in 1967 by Kurita and coworkers.[399] These changes were characterized by trisomy in the C-1 and/or A-6 chromosomes. Earlier researchers reported that DMBA-induced leukemias in mice consistently showed 41 chromosomes in the modal cell lines instead of the normal 40.[400] Sugiyama[401] obtained an apparent relationship between sarcomatogenic potency and the incidence of chromosome breakage in rat bone marrow cells produced by a single intravenous injection (50 mg/kg B.W.) of various benz[a]anthracene derivatives. It was apparent that benz[a]anthracene and several non- or weakly-carcinogenic derivatives (7,12-diethyl-; 7-ethyl-; 12-ethyl-) produced no elevation of chromosome aberrations, whereas the more active carcinogens (7,12-dimethyl-; 6,8,12-trimethyl; 7,8,12-trimethyl) produced aberrant metaphase cells.

Considerable evidence is also available to indicate that chromosome alterations in POM-induced tumors *in vivo* are not consistent either in frequency or in pattern. DMBA-induced tumors (fibrosarcoma, squamous carcinoma, lymphosarcoma) of the uterine cervix in ICR mice revealed various karyotypic compositions.[402,403] These tumors displayed diploid, aneuploid, tetraploid, and octaploid chromosome constitutions. Tumors induced in mice with MCA and dibenzo[a,i]pyrene also showed a wide variation in chromosome constitution.[404,405] Mice treated with 30 µg DMBA, a dose sufficient to produce a 100 percent incidence of thymic lymphomas, did not reveal an excess of chromosome abnormalities in bone marrow or thymus.[406] Even at higher doses (60 µg DMBA), the incidence of abnormal chromosomes did not significantly differ from controls. Subcutaneous tumors in Syrian hamsters induced by single injections of BaP (0.1 µg) or DMBA (0.1 mg), and cultured cell populations derived from these tumors, failed to reveal common karyotypic changes.[407] Tumor cells had subdiploid, diploid, and hypotetraploid chromosome constitutions; further karyotype rearrangements occurred with subsequent growth *in vitro*.

In humans, the presence of the "Philadelphia" chromosome in myeloid leukemia appears to be the only example of a human chromosome abnormality which is tumor-specific.[408] In POM-induced experimental tumors, lymphatic leukemia in mice produced by DMBA also displays consistent chromosome abnormalities.[402] Beyond this common feature, convincing data have not been presented to indicate that somatic cells exposed to POM may suffer characteristic or reproducible damage to the genome. Instead, it is proposed that random karyotypic mutants of transformed cells are selected in response to growth pressures in the host environment (e.g., tissue necrosis, infection, anoxia, lack of nutrition).[402]

Evidence has not been encountered in the published literature concerning the likelihood of POM-induced somatic mutation in the absence of neoplastic transformation.

6.5.2.2 Effects in germinal tissues--The fruit fly, Drosophila melanogaster, is commonly used as a whole animal submammalian genetic test system. Assays can be conducted for gene mutations at specific loci and for small deletions resulting in recessive lethal and visible mutations. Moreover, Drosophila is apparently capable of activating compounds that are not mutagenic or carcinogenic in their administered form.[410] The use of Drosophila has been suggested as a valuable bridge between microbial and mammalian assays.[410]

A series of studies was undertaken by Fahmy and Fahmy[411-414] which demonstrated conclusively that carcinogenic polycyclic hydrocarbons were selectively mutagenic to the male germ cell line of Drosophila. Mutagenicity was restricted to the tRNA and rRNA loci, yielding Minute (M) and bobbed (bb) mutants, respectively. The activity of benz[a]anthracene, 7-methylbenz[a]anthracene, MCA, DMBA, their epoxides, and some other derivatives was determined in tests of both general mutagenicity on the whole genome and specific locus mutability on the tRNA and rRNA genes.[414] The overall yield of sex-linked recessive lethals and visibles (general mutagenicity test) was not significantly enhanced over control levels by the parent hydrocarbons. However, several of the K-region epoxides of these compounds were appreciably mutagenic (Table 6-35). In the

Table 6-35. MUTAGENIC RESPONSE OF THE X CHROMOSOMES IN *Drosophila*[a] 414

Compound	Dose, mM	Gametes tested	Lethals		Visibles		Total X mutations/10^3
			Number	Per 10^3	Number	Per 10^3	
Controls[b]		3707	6(1)[c]	1.6	2	0.5	2.2 ± 0.8
			A. Parent hydrocarbons				
Benz[a]anthracene	1.0	3790	9(2)	2.4	2	0.5	2.9 ± 0.9
	5.0	3473	12	3.5	4(2)	1.2	4.6 ± 1.1
	10.0	1773	7(2)	3.9	3	1.7	5.6 ± 1.8
7-Methylbenz[a]anthracene	5.0	1703	5	2.9	0	0.0	2.9 ± 1.3
	10.0	1709	4(2)	2.3	0	0.0	2.3 ± 1.2
7,12-Dimethylbenz[a]anthracene	1.0	1833	7(3)	3.8	0	0.0	3.8 ± 1.4
	5.0	1753	5(1)	2.9	0	0.0	2.9 ± 1.3
3-Methylcholanthrene	10.0	2399	5(3)	2.1	2(1)	0.8	2.9 ± 1.1
			B. Reactive derivatives				
5,6-Epoxy-5,6-dihydrobenz[a]anthracene	1.0	1639	1(1)	0.6	5(2)	3.1	3.7 ± 1.5
	5.0	1606	12(2)	7.5	4(1)	2.5	10.0 ± 2.5
8,9-Epoxy-8,9-dihydrobenz[a]anthracene	1.0	1588	3	1.9	0	0.0	1.9 ± 1.1
5,6-Epoxy-5,6-dihydro-7-methylbenz[a]-anthracene	5.0	2623	12	4.6	6(1)	2.3	6.9 ± 1.6
	10.0	1709	14(2)	8.2	7	4.1	12.3 ± 2.7
5,6-Epoxy-5,6-dihydro-7,12-dimethyl-benz[a]anthracene	1.0	1725	3	1.7	1(1)	0.6	2.3 ± 1.2
	5.0	1710	11(1)	6.4	0	0.0	6.4 ± 1.9
11,12-Epoxy-11,12-dihydro-3-methylchol-anthrene	5.0	1779	12(1)	6.7	3	1.7	8.4 ± 2.2
	10.0	1695	12(1)	7.1	3	1.8	8.8 ± 2.3
Benz[a]anthracene-cis-5,6-dihydro-5,6-diol	5.0	1891	4	2.1	0	0.0	2.1 ± 1.1
7-Bromomethylbenz[a]anthracene	1.0	1890	5	2.6	1	0.5	3.2 ± 1.3
	5.0	3260	15(1)	4.6	5	1.5	6.1 ± 1.4

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[a] General mutagenicity of various benz[a]anthracenes and their reactive derivatives as indicated by the yield of sex-linked recessive lethals and visibles.

[b] Response with administration vehicle only; 2% dimethylformamide in arachis oil.

[c] Mosaic mutants are entered in parentheses.

specific locus test, DMBA, MCA, and 7-methylbenz[a]anthracene produced significant increases in the yields of phenotypic bb plus M and transmitted bb's, whereas benz[a]anthracene did not induce significant numbers of mutations on the RNA genes (Table 6-36). Thus, mutagenicity was correlated with carcinogenicity, although mutation frequencies did not vary with the dose employed. On the other hand, the mutagenicity of the hydrocarbon epoxides on the RNA genes was apparently dose-related.

It was significant to note that the K-region epoxides of all derivatives tested displayed appreciable nonspecific mutagenicity as evidenced by the high number of X chromosome mutations (lethals and visibles) in comparison to controls and the parent hydrocarbons. An explanation was offered based upon the high reactivity of electrophilic carbonium ions derived from the epoxides which may react nonspecifically with nucleophilic centers in DNA, especially the N-7 of guanine. The fact that the parent hydrocarbons displayed greater mutagenic specificity than their K-region epoxides argued in favor of the involvement of a different reactive metabolite (i.e., non-K-region epoxides, dihydrodiols, or diol-epoxides). Moreover, the correlation of mutagenic activity to RNA genes with carcinogenicity in rodent skin (DMBA>MCA>benz[a]-anthracene) implied a common intracellular target and/or ultimate reactive metabolite.

For the detection of mutagenicity in the whole mammal, the male dominant lethal assay in mice is most commonly employed. However, it is felt that this test is not particularly sensitive.[415] Kennedy and coworkers[416] administered BaP (50 and 100 mg/kg B.W.) and benz[a]anthracene (15 and 30 mg/kg B.W.) by intraperitoneal injection to male albino mice which were subsequently mated to virgin females. The females were sacrificed after one week

Table 6-36. MUTAGENIC RESPONSE OF THE BB LOCI IN *Drosophila*[a] [414]

Compound	Dose, mM	Gametes tested	Phenotypic bb + M		Transmitted bb	
			Number	Per 10^3	Number	Per 10^3
Controls[b]		124,724	156(7)[c]	1.3 ± 0.1	45(1)	0.4 ± 0.1
A. Parent hydrocarbons						
Benz[a]anthracene	1.0	44,314	49(13)	1.1 ± 0.2	6(1)	0.1 ± 0.1
	5.0	36,854	73(10)	2.0 ± 0.2	24(4)	0.7 ± 0.1
	10.0	9,986	14(1)	1.4 ± 0.4	9(5)	0.9 ± 0.3
7-Methylbenz[a]anthracene	5.0	7,798	34	4.4 ± 0.7	9(3)	1.2 ± 0.4
	10.0	10,804	52(5)	4.8 ± 0.7	14(1)	1.3 ± 0.3
7,12-Dimethylbenz[a]anthracene	1.0	29,204	259(7)	8.8 ± 0.5	182(4)	6.2 ± 0.5
	5.0	16,887	134(8)	7.9 ± 0.7	97(8)	5.7 ± 0.6
3-Methylcholanthrene	10.0	18,263	86(5)	4.7 ± 0.5	62(5)	3.4 ± 0.4
B. Reactive derivatives						
5,6-Epoxy-5,6-dihydrobenz[a]anthracene	1.0	14,426	27(4)	1.9 ± 0.4	1(1)	0.1 ± 0.1
	5.0	4,626	7(2)	1.5 ± 0.6	1	0.2 ± 0.2
8,9-Epoxy-8,9-dihydrobenz[a]anthracene	1.0	16,078	23(4)	1.4 ± 0.3	3(2)	0.2 ± 0.1
5,6-Epoxy-5,6-dihydro-7-methylbenz[a]-anthracene	5.0	32,468	56(7)	1.7 ± 0.2	18(3)	0.6 ± 0.1
	10.0	7,828	28	3.6 ± 0.7	12(2)	1.5 ± 0.4
5,6-Epoxy-5,6-dihydro-7,12-dimethyl-benz[a]anthracene	1.0	23,660	44(2)	1.9 ± 0.3	18(1)	0.8 ± 0.2
	5.0	6,356	38(2)	6.0 ± 1.0	29(4)	4.6 ± 0.8
11,12-Epoxy-11,12-dihydro-3-methylchol-anthrene	5.0	19,326	25(1)	1.3 ± 0.3	18	0.9 ± 0.2
	10.0	30,995	63(4)	2.0 ± 0.3	28(1)	0.9 ± 0.2
Benz[a]anthracene-cis-5,6-dihydro-5,6-diol	5.0	19,870	26(5)	1.3 ± 0.3	5(2)	0.3 ± 0.1
7-Bromomethylbenz[a]anthracene	5.0	21,233	26(3)	1.2 ± 0.2	4(2)	0.2 ± 0.1

[a] The yield of transmissible bb's among the phenotypically scored bristle mutations with various benz[a]anthracenes and their reactive derivatives.

[b] Response with administration vehicle only; 2% dimethylformamide in arachis oil.

[c] Mosaic mutants are entered in parentheses.

and the mutation rate determined by counting the numbers of fetal resorptions versus the total number of implantations. These investigators found no evidence of genetic damage which might have been manifested as a dominant lethal mutation.

In contrast to these results, Epstein and coworkers[417] observed an excess incidence of dominant lethal mutations in ICR/Ha Swiss mice using high doses of BaP and MCA (Table 6-37). The authors noted a lack of consistency in the results obtained, both in terms of dose (for MCA) and in magnitude of response and parameters affected (for BaP). Nevertheless, in control animals the mean weekly pregnancy rate was 66 percent, the mean number of total implants per pregnancy was 11.5 to 11.9, and the mean early fetal deaths per pregnancy were almost always less than 0.95. Thus, considerable differences are evident between POM-treated groups and untreated controls.

Additional evidence which may indicate a potential for POM-induced mutagenesis in germinal tissues was provided by Wyrobek and Bruce.[418] They found that five daily intraperitoneal injections of BaP or MCA at doses up to 100 mg per day caused abnormally shaped sperm in (C57BL x C3H)F_1 mice. These effects were most pronounced at four and ten weeks after treatment, thereby indicating damage to the primary spermatocytes and spermatogonia. Since abnormalities in sperm morphology were found, these results may be indicative of mutagenesis in the male germ cell line.

6.5.3 Correlation of Mutagenicity with Carcinogenicity

The recent widespread use of _in vitro_ mutagenicity bioassay systems (e.g., Ames assay) to predict the carcinogenic activity of chemical pollutants and complex environmental mixtures has led to considerable controversy over the reliability of obtained results. In particular, the practice of

Table 6-37. AGENTS PRODUCING EARLY FETAL DEATHS AND/OR PREIMPLANTATION LOSSES SIGNIFICANTLY BEYOND CONTROL LIMITS BUT REQUIRING FURTHER VERIFICATION BECAUSE OF LACK OF INTERNAL CONSISTENCY[417]

	Route and frequency of administration	Total weeks of mating	Doses, mg/kg	No. of males (No. of deaths)	Parameters selected in screen (mating weeks)				Comments
					% Pregnancy	Implants/ pregnancy	ED[a]/pregnancy	% Pregnancy with ED	
nzo[a]pyrene	ip	3	500	5(1)	25(2)	---	---	---	Lack of consistency between experiments
			1000	5	13(3)	7.5(3)	1.00(1);1.00(2)	60(1);67(2)	
	ip	3	750	14	---	---	---	---	
			1000	14	29(3)	---	---	53(3)	
	ip	8	750	7(4)	<30(4-8)	7.6(3);6.3(4)	---	---	
			1000	9(2)	<30(4-8)	4.0(6)	---	---	
20-Methylcholanthrene	ip	8	100	7(2)	19(1)	---	---	---	Dose reversal in other weeks
			200	9(1)	---	8.2(7)	---	---	

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[a] ED, early deaths.

extrapolating from data regarding in vitro mutagenic potency to predict in vivo carcinogenic potency has often been criticized. It now appears that these criticisms are at least partially justified in the case of POM.

In a recent paper published by Andrews and coworkers[419] 24 POM were tested for mutagenicity using the Ames Salmonella assay in the presence of Aroclor 1254-stimulated rat liver microsome S9 mix. Tester strains TA1535, 1537, 1538, 98, and 100 were employed using the plate incorporation method and liquid culture assay. Among the 24 compounds tested, mutagenicity was correlated with carcinogenicity for 14 (58 percent), while there was no correlation for 10 (41 percent) of the compounds (Table 6-38). Of the compounds showing a negative correlation, eight were false positives and two were false negatives. Difficulty in the interpretation of the false positive results arises from the fact that information concerning carcinogenicity may be incorrect (e.g., BeP is considered as noncarcinogenic by the authors, but as a weak carcinogen by others). Interpretation of the false negative results, on the other hand, is considerably more difficult.

One of the most likely explanations for the lack of qualitative agreement between Ames assay and carcinogenicity bioassay results is the type of metabolic activation system employed for mutagenesis testing. Variables such as the choice of organ from which the S9 mix is prepared, the choice of species, the choice of enzyme-inducing agent, and the volume of S9 mix employed are all believed to influence the outcome of a particular experiment.[420,421] For example, Tang and Friedman[422] demonstrated that while human liver enzymes were effective in activating aflatoxin B_1 to a mutagen in the Ames assay, they were highly variable in activating aromatic amines,

Table 6-38. COMPARISON OF MUTAGENICITY IN AMES ASSAY WITH CARCINOGENICITY OF POLYNUCLEAR HYDROCARBONS[419]

Test Chemical	Carcinogenic	Mutagenic
dibenz[a,j]anthracene	+	+
5,6-dihydrodibenz[a,j]anthracene	+	+
1,2,3,4,8,9-hexahydrodibenz[a,j]-anthracene	-	+*
dibenz[a,h]anthracene	+	+
1,2,7,8-tetrahydrodibenz[a,h]-anthracene	-	+
5,6-dihydrodibenz[a,h]anthracene	+	+
7,14-dihydrodibenz[a,h]anthracene	+	+
1,2,3,4,12,13-hexahydrodibenz[a,h]-anthracene	+	+
dibenz[a,c]anthracene	+	+
9,14-dihydrodibenz[a,c]anthracene	?	+
10,11,12,13-tetrahydrodibenz[a,c]-anthracene	-	+
7,12-dimethylbenz[a]anthracene	+	+
5,6-dihydrodimethylbenz[a]-anthracene	+	-**
8,9,10,11-tetrahydrodimethylbenz-[a]anthracene	+	-**
anthanthrene	+	+
4,5-dihydroanthanthrene	-	+
1,2,3,7,8,9-hexahydroanthanthrene	-	+
3-methylcholanthrene	+	+
11,12-dihydromethylcholanthrene	+	+
6,7,8,9,10,12b-hexahydromethyl-cholanthrene	+	+
benzo[ghi]perylene	-	+
5,6,7,8,9,10-hexahydrobenzo[ghi]-perylene	-	+
9,10-dimethylanthracene	+	+
benzo[a]pyrene	+	+
benzo[e]pyrene	-	+

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* Only in liquid test

** Not mutagenic in plate or liquid test and generally ineffective with BaP or MCA. In a comparison of BaP metabolism in liver microsomes (rat and hamster), hamster embryo cell microsomes, and intact hamster embryo cells, significant differences were noted in the formation of metabolic products.[423] In particular, the author showed how cell disruption during preparation of microsome-containing mixtures leads to altered metabolite ratios which could have a significant impact on the outcome of mutagenesis assays that employ such mixtures. The author concluded that the use of intact cell-feeder layers to provide metabolic activation for in vitro bioassays should lead to more reliable results for in vivo comparisons.

A comprehensive investigation by LaVoie and coworkers[424] has recently provided a uniform basis for the comparison of mutagenicity with carcinogenic activity for various POM. They examined both qualitative and quantitative differences in complete carcinogenicity, tumor-initiating activity, and mutagenic response in the Ames assay for compounds tested under identical experimental conditions. In all, 40 compounds were tested which contained four or more fused ring systems. These included: chrysene and its methyl- and fluoro-derivatives; fluoranthene and its methylated derivatives; benzofluoranthenes; benzopyrenes; perylene; dibenzopyrenes; naphtho[2,3-e]pyrene; indeno[1,2,3-cd]pyrene; benzo[ghi]perylene; and pyrene. The Ames Salmonella/microsome assay was 96 percent (26 of 27) effective in detecting positive carcinogens, and 36 percent (4 of 11) effective in detecting negative carcinogens. In addition, one false negative result (6-methylchrysene) was obtained. Data from the tumor initiation bioassay indicated that it is a better predictor of carcinogenic potential than the Ames Salmonella/microsome assay system. Furthermore, quantitation of the mutagenic response among structurally related POM in Salmonella did not accurately reflect either tumor-initiating activity or complete carcinogenicity.

These results have important implications for the use of the Salmonella/microsome mutagenicity assay system in evaluating carcinogenic hazards. Most important, the authors concluded that the mutagenicity of POM mixtures should not be equated with carcinogenic hazard in the absence of analysis of relative composition, and quantitative comparisons of mutagenic potency for POM do not provide a reliable indication of relative carcinogenic potential.

As an illustration of the misleading results which can be obtained with bacterial mutagenicity assays of complex environmental mixtures, Andrews and coworkers[419] considered the situation with petroleum and coal tar products. These substances have a high content of benzo[ghi]perylene relative to BaP content. However, since both compounds show nearly equal mutagenic activity in Salmonella, an incorrect assumption concerning carcinogenic potential of the mixture could result.

The use of mammalian mutagenicity bioassay systems may also lead to erroneous predictions regarding carcinogenic activity of POM, although large numbers of compounds have not yet been tested in any one system. Among the cytogenetic assays which are currently being used for screening of environmental chemicals, the sister-chromatid exchange (SCE) test is regarded as being more sensitive than the test for induction of chromosomal aberrations. A comparison has been made of the ability of eight POM to induce SCE in Chinese hamsters bone marrow cells by intraperitoneal injection.[425] Their results, which are summarized in Table 6-39, revealed a poor correlation between mutagenicity and carcinogenicity for several of the compounds tested.

Table 6-39 . INDUCTION OF SISTER-CHROMATID EXCHANGES BY INTRAPERITONEAL INJECTION OF POM IN CHINESE HAMSTERS [425]

Treatment 2 X 450 mg/kg	Animals (n)	Metaphases (n)	SCEs/metaphase mean ± SD	t test
control	8	400	3.9 ± 0.9	--
anthracene	8	400	3.7 ± 0.8	--
dibenz[a,h]anthracene	8	400	4.9 ± 0.7	$p<0.02$
chrysene	8	400	5.1 ± 1.0	$p<0.02$
phenanthrene	8	400	5.5 ± 0.7	$p<0.01$
benzo[e]pyrene	8	400	5.5 ± 0.7	$p<0.01$
benzo[b]fluoranthene	8	400	5.6 ± 0.5	$p<0.01$
benzanthracene	8	400	6.1 ± 0.4	$p<0.01$
benzo[a]pyrene	8	400	10.6 ± 1.6	$p<0.01$

Phenanthrene (a noncarcinogen) and benzo[e]pyrene (inactive to weakly-active carcinogen) both induced more SCE's than DBahA (a strong carcinogen).

6.6 REPRODUCTION AND TERATOLOGY

The interference with reproductive success by exposure to POM has only been documented for a few compounds. When large doses (0.5 to 5.0 mg) of MCA in olive oil were injected in mice on the first or seventh day of pregnancy, fetal resorption usually occurred in most of the treated animals.[426] However, sham-treated controls were not included in the study, thereby making interpretation of the results quite difficult. Treatment with MCA at mid-term (day 11) was ineffective in interrupting pregnancy (Table 6-40). Among those animals going to full term and giving birth, the offspring displayed reduced body weights in comparison to controls but no congenital abnormalities were evident at birth or during a 3-month observation period. Although similar treatment of non-pregnant mice with MCA had no apparent effect, it is difficult to determine whether pregnancy was interrupted by a direct action on the embryo. This is especially difficult in light of evidence which indicates that MCA does not readily cross the placenta (see Section 6.1).

Subsequent studies did establish, however, that if MCA directly contacts mid-term mouse embryos, fetal resorptions and malformations will occur.[427] In these experiments, 0.01 ml of a 0.025 percent solution of MCA in benzene was injected directly into the embryonic fluids of 10-day-old C3H(Jax) mouse embryos. Their results, presented in Table 6-41, indicate that fetal resorptions and malformations were very common. From the data presented, it is not possible to determine the contribution of the benzene injection vehicle in producing the embryopathic effects observed. Moreover, a 9.3 percent incidence of fetal resorptions in sham-operated (incisions made but no MCA or vehicle injected) control mice was observed, which could not be attributed to a specific cause.

Table 6-40. EFFECT OF 3-METHYLCHOLANTHRENE ON PREGNANCY IN MICE[426]

Route of administration	Duration of pregnancy, days	Total dose of 3-methylcholanthrene, mg	Number of animals in which pregnancy was interrupted / Total number studied
Intraperitoneal	1	0.5	4/5[a]
	1	1.0	7/7[a]
	6-7	1.0	2/3
	11	1.0	0/3
Subcutaneous	1	2.0	3/5[a]
	1	5.0	11/11[a]
	6-7	1.0	3/5
	6-7	2.0	3/4
	6-7	5.0	6/6
	11	2.0	0/2
	11	5.0	0/4
Subcutaneous	7	2.0	2/5[b]
	7	5.0	7/7[b]

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[a] A total of 50 mice, in which vaginal plugs were found, received no treatment; 39 of these animals became pregnant.

[b] Mice ovariectomised and received 1.5 mg of progresterone per day.

Table 6-41. DATA ON MOUSE EMBRYOS TREATED WITH 0.01 ml OF 3-METHYLCHOLANTHRENE[427]

Group	Number pregnant animals	Number breeders that delivered	Number embryos treated	Number young born	Prenatal mortality, %	Hemorrhages	Tail defects	Other defects	Total	Abnormalities, %
0.025% Methylcholanthrene in benzene[a]	19	10	122	34	72.1	7	5	2	14	41.2
0.025% Methylcholanthrene in benzene[b]	10	4	67	19	71.7	4	2	4	10	52.6
Sham-operated control	6	6	43	39	9.3					0.0

[a] Offspring delivered normally.

[b] Offspring delivered by Caesarean section.

Little success has been achieved in demonstrating a marked effect of BaP on reproduction, despite its ease of absorption and extensive tissue distribution (including transplacental). Early studies involved the feeding of BaP to dogs, rats, mice, chickens, ducks, and cockroaches and indicated that a period of infertility may occur in the chicken, and fetal resorptions may occur in the rat.[428] Rats fed 1.0 mg BaP per gram of food displayed no abnormalities in the ovarian cycle, ovulation, fertilization, and implantation; a few resorptions were observed, however. A more detailed investigation was subsequently pursued to establish if lower doses of BaP might affect fertility and development in Swiss mice.[429] At levels of 0.25 mg or 0.50 mg BaP per gram of food given either before, during, or after mating, no adverse effects could be shown on fertility or the developing embryo. Even when BaP concentrations were increased to 1.0 mg per gram of food, mice continued to reproduce normally. Neither males nor females were affected by this ration. Mice fed BaP in their diet at 1.0 mg per gram of food since the time of weaning appeared normal and gained weight readily.

Although the effects of BaP on reproductive function are apparently not severe, a transplacental carcinogenic action has been shown with very large doses of BaP.[430] Mice of the Ha/ICR strain were administered BaP (4 mg) in carbowax-400 subcutaneously on days 11, 13, and 15 of pregnancy. Offspring were delivered by Caesarean section and nursed on untreated foster mothers to ensure that no BaP exposure could occur through the milk. After weaning at 4 weeks of age, mice received twice weekly applications of 1 percent croton oil in acetone dropped on the back to determine if BaP-initiated tumors could be promoted. Mice were sacrificed at age 28 weeks and examinations made for the formation of pulmonary adenomas. As the data in Tables 6-42 and 6-43 indicate, prenatal treatment with BaP considerably enhanced tumor development in both lung and skin during the postnatal period.

In contrast to the questionable teratogenic effects produced by MCA or BaP, DMBA and its hydroxymethyl derivatives possess considerable teratogenic potency.[431,432] Treatment of Sprague-Dawley rats with a single intravenous dose of 7-hydroxymethyl-12-methylbenz[a]anthracene (about 5.5 to 7.0 mg) in olive oil on day 8 of pregnancy produced 100 percent fetal resorptions. A similar dose of DMBA on day 8 of pregnancy produced a smaller incidence of fetal resorptions. Resorptions in control rats given olive oil with or without saline averaged 7 percent (maximum 9 percent). Treatment with 7-hydroxymethyl-12-methylbenz[a]anthracene on day 12 to day 14 of pregnancy resulted in malformations in every surviving fetus. Characteristic pathology was found in all littermates: stunting, lordosis of cervical and upper thoracic parts of the vertebral column, and an encephalocele and a spina bifida. No skeletal deformities were seen among 2000 controls. Teratogenic effects in the absence of an increased resorption rate were also obtained with DMBA given on day 13 of pregnancy. However, these effects were not as pronounced as those produced by the hydroxymethyl derivative. Since 7-hydroxymethyl-12-methylbenz[a]anthracene is a principal metabolite of DMBA and also exceeds the parent compound in teratogenic potency, it was postulated that metabolic activation precedes fetal damage. Furthermore, it was proposed that the ultimate embryopathic substance derived from DMBA may be a metabolite of 7-hydroxymethyl-12-methylbenz[a]anthracene, since teratogenesis could be inhibited by pretreatment of mice with microsomal enzyme inhibitors before administration of the

Table 6-42. EFFECT OF BENZO[A]PYRENE ADMINISTRATION TO PREGNANT MICE ON PULMONARY ADENOMA FORMATION IN THE PROGENY[430]

Experimental group	Material administered	No. of mice	No. of mice with adenomas[a]	% of mice with adenomas	No. of adenomas per group	No. of adenomas per mouse	No. of mice with multiple adenomas	
							3-6	7-9
Absolute controls	Nothing	82	6	7.3	7	0.09	0	0
Vehicle controls	Carbowax-400	89	10	11.2	13	0.15	1	0
Benzo[a]pyrene	Benzo[a]pyrene in carbowax-400	55	34	61.8	130	2.36	13	6

[a] Number of mice with pulmonary adenomas at 28 weeks of age.

Table 6-43. EFFECT OF BENZO[A]PYRENE ADMINISTRATION TO PREGNANT MICE ON SKIN PAPILLOMA FORMATION IN THE PROGENY[430]

Experimental group	No. of mice	No. of mice with papillomas[a]	% of mice with papillomas	No. of papillomas per group	No. of papillomas per mouse
Absolute controls	82	6	7.3	8	0.10
Vehicle controls	89	8	9.0	9	0.10
Benzo(a)pyrene	55	13	23.6	18	0.33

[a] Number of mice with skin papillomas after 24 weeks of topical treatment twice weekly with 1% croton oil. Mice were 28 weeks of age.

hydroxymethyl compound.[432] Unfortunately, the use of large doses of DMBA and its derivatives precludes the possibility of risk assessment under realistic environmental exposure situations.

6.7 HUMAN STUDIES

Epidemiological evidence on the health effects of POM comes from both occupational and community air pollution studies. The effects of ingested POM on humans have been largely ignored, despite the fact that this is potentially the most significant route of exposure in non-tobacco smokers. The absence of health effects data on POM ingestion is the most important information gap in the present epidemiology data base.

Occupational studies, especially among gas production workers and coke plant workers, have shown that long-term exposure to the products of the destructive distillation of coal is associated with an elevated rate of lung cancer as well as cancer of the digestive tract and other sites (e.g., pancreas, kidney). Community studies, which involve much lower levels of inhalation exposure and are more problematic because of the heterogeneous populations involved, attempt to detect an association between community morbidity and mortality rates and some direct or indirect index of air pollution. These studies have shown, in certain cases, an excess of respiratory cancers and malignancies of other sites (e.g., stomach, prostate) associated with elevated POM levels in urban settings.

In both types of studies, inhalation of pollutants is not limited to POM alone but includes exposure to irritant gases such as SO_2 and NO_x, particulate matter, and trace metals (e.g., arsenic, nickel, chromium) which may exert their own effects or may enhance the effects of the POM. Additionally, in both occupational and nonoccupational air pollution studies, smoking is a powerful causative factor of similar diseases.[433,434] In community air pollution studies, occupational exposure represents another potential causative employed in occupations with high levels of exposure to toxic substances.[435]

It is tempting to speculate that cancers of the non-respiratory tissues associated with airborne POM are not in fact the result of POM absorption via the respiratory tract. It is known that POM are adsorbed to particulate matter in air, and that POM-containing particulates may be rapidly cleared from the lungs by mucociliary action and subsequently swallowed. Thus, it is conceivable that significant ingestion of POM occurs secondary to the inhalation of POM adsorbed to particulates. However, the anticipated ingestion of POM via particulates in air would constitute only a minor increment relative to POM ingestion from foods. Therefore, the more relevant factor to consider in evaluating the role of POM in non-respiratory cancers is the dietary contribution to total POM exposure. Unfortunately there has been no attempt in either occupational or community studies to separate dietary from airborne sources of POM.

6.7.1 Occupational Studies

In occupational situations the effects of long-term exposure to high levels of a toxicant are more easily quantified than in community settings, thus allowing for extrapolation back to the effect of small doses received via the ambient atmosphere. The most extensive epidemiological studies of occupational groups with exposure to POM are those of coke plant workers and gas production workers. Other relevant studies include those of wax pressmen and roofers.

Epidemiological studies in different countries have demonstrated that workers exposed to the products of the combustion and distillation of bituminous coal experience an increased incidence of cancer of several sites (lung, pancreas, kidney, bladder, skin). These studies are discussed below, and the overall results are summarized in Tables 6-44 and 6-45.

The earliest association of skin cancer with occupations involving exposure to coal-combustion products was that of Percival Pott, who in 1775 observed a high incidence of scrotal cancer among chimney sweeps exposed to soot. His observation has now become a classic reference of occupational medicine for cancer and for discussions of coal tar products. In the early 20th century, several studies established the association between the handling of coal tar products and pitch products and skin cancer.[436,437] Kennaway and Kennaway, in a later series of reports, found an increased rate of bladder and lung cancer in occupations involving exposure to coal gas, tar, pitch, and soot.[438,439,440]

In these studies, the number of workers in any one occupational group was small, and it was not possible to calculate relative risks for exposed groups with certainty or to obtain evidence for a dose-response relationship.

Kuroda[441] demonstrated a high incidence of lung cancer among Japanese gas generator workers. Although lung cancer was a relatively rare form of cancer in Japan during the 1930's (accounting for only 3.1 percent of all cancer), this study showed that lung cancer accounted for 80 percent of all cancer among the gas generator work force who were exposed to extremely high quantities of coal-tar-pitch volatiles.

In a more recent study of 504 deaths among former gas workers at a Japanese steel plant, Kawai et al.[442] found six deaths from lung cancer in contrast with the expected number, 0.180, using other workers at the same

Table 6-44. SUMMARY OF EPIDEMIOLOGICAL EVIDENCE OF CARCINOGENICITY

Investigator	Date of study	Type of study	Site	Results	Characterization of exposure
Kuroda and Kawahata	1936	Historical prospective	Lung	(12 lung cancer deaths out of a total of 15 deaths for all cancers) Relative risks cannot be calculated but there is suggestion of 26-fold excess over general population (Lloyd, 1971)	Producer gas workers
Kawai, Amamoto and Harada	1967	Historical prospective	Lung	33 times the rate for other steelworkers	Workers in producer gas works
Henry, Kennaway and Kennaway	1931	Historical prospective	Bladder	1-1/2 to 4-fold increased risk relative to general population	Workers in 5 out of 10 occupations involving exposure to coal products
Kennaway and Kennaway	1936	Historical prospective	Lung	3-fold excess for "gas stokers and coke-oven chargers"	British producer gas workers; chimney sweeps and several categories of gas-works employees
Kennaway and Kennaway	1947	Historical prospective	Lung		
Doll	1952	Historical prospective	Lung	81% excess of lung cancer deaths	Former gas retort workers (pensioners)
Doll	1965	8-year prospective	Lung	Those with heavy exposure showed 69% excess relative to those with minimal exposure (by-products workers)	Gas-worker mortality was greatest for those with greatest exposure (retort house workers)
			Bladder	Those with heavy exposure showed 4-fold excess relative to those with minimal exposure	
Doll	1972	4-year follow-up of 8-year prospective	Lung	Heavily exposed workers showed highly significant excess; by-products workers showed no excess	

Table 6-44 (continued). SUMMARY OF EPIDEMIOLOGICAL EVIDENCE OF CARCINOGENICITY

Investigator	Date of study	Type of study	Site	Results	Characterization of exposure
Reid and Buck	1956	Retrospective study of deaths among coke plant workers during 1949-1954	Lung	No excess in number of cancer deaths among coke plant workers as a whole nor of lung cancer for oven workers	Coke plant workers
Brunsgaard	1959	Retrospective study of deaths among employed and retired gas-workers over a 15-year period	Lung	6.4-fold increase over general population	All deaths occurred among gas-workers with at least 5 years experience; most had more than 10 years
			Bladder	5 deaths that appeared to be a significant excess	
Lloyd, Lundin, Redmond, and Geiser	1970	Prospective study of mortality among steelworkers over work-area	Lung	Coke plant workers had 2-fold excess relative to rate among steelworkers	Coke plant workers
Lloyd	1971	Prospective study	Lung	Coke-oven workers showed 2-1/2-fold excess over steelworker population;	Coke plant workers
			Pancreas	Top-side workers had 5-fold risk; top-side workers with 5 years full-time	
			Intestine	Top-side had 10-fold risk; nonoven workers may have excess risk of digestive cancer	

Table 6-44 (continued). SUMMARY OF EPIDEMIOLOGICAL EVIDENCE OF CARCINOGENICITY

Investigator	Date of study	Type of study	Site	Results	Characterization of exposure
Redmond, Ciocco, Lloyd, and Rush	1972	Follow-up to prospective study	Lung	Coke oven workers had 1.34 excess risk relative to nonoven workers	Coke plant workers
			Kidney	Coke oven workers had a 7.49 excess risk over nonoven workers	
			Prostate	Coke oven workers had 1.64 excess risk over nonoven workers	
Redmond	1976	Follow-up to previous study	Lung	15.72 excess for full-time top-side oven workers	
			Intestine	2.37 excess for nonoven workers	
			Pancreas	4.29 excess for nonoven workers	
Redmond et al.	1976	Historical prospective of by-products workers	Kidney	5 excess for all coke plant workers;	
			Lung	3.31 excess for oven workers	
			Pancreas	4.95 excess for nonoven workers	
			Intestine	2.93 excess for nonoven workers	
			Buccal Pharyngeal		

Table 6-45. SUMMARY OF OBSERVATIONS ON OBSERVED DEATHS AND RELATIVE RISKS OF DEATH FROM ALL CANCERS, RESPIRATORY CANCER, AND CANCER OF THE DIGESTIVE TRACT[a]

Length of employment, yr	Distribution of workers			Deaths and RR's of death from malignant neoplasms						Deaths and RR's of death from respiratory cancer					
	5+	10+	15+	5+		10+		15+		5+		10+		15+	
				Obs.	RR	Obs.	RR	Obs.	RR	Obs.	RR	Obs.	RR	Obs.	RR
Total coke oven	1860	1194	790	166	1.47[b]	136	1.50[b]	108	1.62[b]						
Coke oven	993	574	325	101	1.66[b]	85	1.95[b]	63	2.40[b]	54	3.02[b]	44	3.42[b]	33	4.14[b]
Oven topside full-time	150	72	29	35	3.70[b]	22	5.12[b]	12	7.63[b]	25	9.19[b]	16	11.79[b]	8	15.72[b]
Oven topside part-time	290	245	159	26	1.59[b]	31	1.85[b]	32	2.73[b]	12	2.29[b]	16	3.07[b]	18	4.72[b]
Oven side only	553	257	137	40	1.17	32	1.46	19	1.51	17	1.79[c]	12	1.99[b]	7	2.00
Nonoven	836	578	392	65	1.28	48	1.10	39	1.13						
No one coke plant area	31	42	73	0	d	3	d	6	1.34						

	Deaths and RR's of death from cancer of digestive system among nonoven workers					
	5+		10+		15+	
	Obs.	RR	Obs.	RR	Obs.	RR
All malignant neoplasms of digestive system	28	1.58[c]	23	1.53	19	1.53
Large intestine	11	2.31[c]	10	2.52[b]	8	2.37[c]
Pancreas	8	3.67[b]	7	3.75[b]	6	4.29[b]
Other	9	0.83	6	0.65	5	0.65

[a] Redmond[451]
[b] P < 0.01.
[c] P < 0.05.
[d] less than 5 deaths.

plant with no gas generator work experience as a control. Age-standardized mortality from lung cancer in the control group was close to that of the general male population. The large excess of lung cancer deaths among the gas workers could not be attributed to smoking. The authors noted that the excess of lung cancer mortality occurred only in the age group of 45 to 54 years. Data for those in this group with 10 to 19 years of gas generator work experience showed a marked increase in lung cancer risk, whereas data for those under 45 years of age having the same work experience (10 to 19 years) showed no significant excess mortality.

Bruusgaard[443] studied 125 deaths among former gas works employees in Norway, all of whom had at least five years' work experience and most of whom had more than ten years. The number of respiratory cancers was higher than expected (12, or 1.6 percent of the total number of deaths, against 1.5 percent in males for the country as a whole). The proportion of lung cancers to cancer of all sites among the gas workers (29.8 percent) was also significantly higher than that in the general population, 9.2 percent. In addition, there were five deaths from cancer of the bladder--12 percent of all cancers. Although Bruusgaard gives no exposure data, and occupational histories for most cases are incomplete, he notes that workers with a history of employment in the retort houses had an especially high incidence of respiratory cancer.

Reid and Buck[444] conducted a mortality study in 1956 among 800 coke plant workers randomly selected from a total of 8000 employed over the years 1949-1954, inclusive. The study did not show an elevated cancer risk when death rates for all causes and for cancer were compared with age-specific rates prevailing in the period 1950-1954 among workers in a large unspecified industrial organization. The cause of death was ascertained either by reference to the union's funeral fund records, which were required to be supported by a copy of the death certificate, or by a special search at the General Register Office. The coke plant workers were categorized by occupation: coke oven workers, those handling by-products, and maintenance workers (further grouped as laborers, workers, and foremen). No total excess in the number of cancer deaths was found among the coke plant workers as a whole, and there was a "complete lack" of any excess of respiratory cancer for men working on the ovens. When occupational history was taken into account, no excessive cancer risk was found for by-product workers and only a small excess was found for men who had at some time worked at the oven.

This study was criticized by Lloyd,[445] who pointed out that Reid and Buck may have underestimated the number of lung cancer deaths, since the records included only men dying while still "on the books" during the period 1949-1954. Lloyd also states that "the population at risk and the distribution by age and area of prior employment were based on an estimate of figures which excluded retirees and those who had left employment."

In an effort to further quantify the Kennaway and Kennaway data suggesting a correlation between occupational exposure and cancer mortality, Doll[446] studied the mortality among male pensioners (over age 60) of a large London gas works company for a 10-year period (1939-1948) and compared the data with mortality data for the population of Greater London. Men who retired early were included in the study on reaching 60 so as not to bias the investigation by the exclusion of a particularly unhealthy group who retired early because of health reasons. Age-standardized mortality ratios were calculated by use of mortality rates for England and Wales, which were weighted to approximate higher rates in Greater London. The causes of death recorded by the company had been copied from death certificates. The pensioners' mortality from all causes was close to the expected (840 deaths against 856 expected), but the mortality from cancer was in excess of the expected (156 against 123.5; $p<0.01$); cancer of the lung accounted for the greatest excess (25 against 10.4; $p<0.001$) which constitutes a significant increase in mortality.

To assess differences in risk among different jobs within the gas works, Doll categorized the pensioners as those employed outside the works and those involved directly in the production of gas or in handling of the waste products, representing a low- and a high-exposure group, respectively. Excess lung cancer among the high-exposure group was significant (17 observed versus 8.6 expected, $0.01<p<0.02$).

In a separate study, Doll[447] carried out an 8-year prospective analysis of mortality from different causes among several occupational groups of gas workers and retirees covering the years 1953-1961. The study included 11,499 men between 40 and 65 years of age at the start of the study with five or more years in the gas works plant. Observed gas worker mortality rates were compared with those expected in populations of England and Wales and regional metropolitan areas.

The workers were grouped into three classes according to their exposure: heavy exposure, intermediate exposure, light exposure, or exposure only to by-products. Again, elevated mortality was attributed to respiratory system disease, specifically, cancer of the lung and bronchitis. The lung cancer mortality rate was 69 percent higher for the heavy exposure group than for the low exposure group. A fourfold higher rate of bladder cancer observed in the heavy exposure group as compared with the light exposure group verged on significance (p = 0.06) according to Doll. He concluded that the mortality of gas workers varied significantly with the type of work and that mortality was highest among workers with greatest exposure to the products of coal carbonization.

A report on an additional four years of observation of the cohort[448] provided follow-up information on 2,449 coal-carbonizing process workers and 579 maintenance workers for mortality rates gathered at annual intervals from 1961 to 1965. Additional employees of four other gas boards were also followed over periods of 7 to 8 years. Cause of death in the original studies was obtained from death certificates. The new data showed a pattern similar to that of data for the first eight years.

Heavily exposed workers experienced a highly significant elevated mortality from lung cancer ($p<0.001$) and bronchitis ($p<0.001$). Data on by-product workers showed no excessive mortality and over the 12-year period provided no substantial evidence of increased occupational risk for this group.

The additional four years of data in this study support the earlier association between exposure to the products of coal carbonization and increased lung cancer and also a risk of bladder cancer (p = 0.06).

The long-term study of mortality among steel workers conducted by Lloyd[445] and Redmond and coworkers[449] has confirmed and extended the well-established findings that workers exposed to the coal-carbonization process experience a markedly increased cancer risk. The successive phases of this study also showed increasing cancer response rates with increasing exposure and dose.

The coke plant workers studied by Lloyd were employed in by-product coke plants. Exposure to effluents from by-product coke ovens is due to the escape of volatiles during charging, discharging, escape through improperly sealed openings, and quenching.

In these studies, the workers were classified by work area within the plant in terms of function and exposure to effluents. The by-product coke plant was analyzed in terms of three distinct areas: (1) the coal-handling area, (2) the coke oven area, and (3) the by-products plants for recovery of gas and chemical products. Since earlier work (e.g., Doll,[448] Kennaway and Kennaway[438,439]) had shown no apparent increased cancer risk for men involved in work similar to that performed in areas (1) and (3), some of the initial study groups included only those workers employed in area (2).

In the first phase of this long-term study, Lloyd[445] undertook a 9-year prospective analysis of 2,552 coke plant workers employed in 1953. He examined the mortality records of the workers in relation to length of employment and work area within the coke plant and compared the cause-specific mortality of coke plant workers as a whole with the mortality of the total steelworker population of 58,828 workers. Coke plant workers were categorized as oven workers and non-oven workers. The excess lung cancer mortality among coke plant workers indicated that risk was elevated nearly threefold among coke oven workers (20 observed deaths versus 7.5 expected). Men working on the tops of the coke ovens had nearly a fivefold increased risk, and men employed five or more years at full-time topside jobs had a tenfold risk (15 observed deaths versus 1.5 expected). Furthermore, a significant excess of cancers of the digestive system was observed in both long- and short-term non-oven workers (17 versus 9.7 expected, significant at the 5 percent level). Cancer of the pancreas and large intestine accounted for the greatest excess.

Redmond et al.,[449] in a follow-up of earlier reports in the series, examined the mortality records of cohorts of coke oven workers in an expanded study at 12 steel plants. In addition, the data from the earlier study[445] of two Allegheny County steel plants with coke plants were updated from 1965 to 1966 and were compared with data from 10 other plants for the same period. The cohorts at the 10 additional plants included all men who had worked at or on the oven at any time in the 5-year period, 1951 through 1955. (The criterion for inclusion in the prior Allegheny County study was employment in one of seven steel plants during 1953).

The findings of Redmond et al.[449] further indicate that both the level and duration of exposure to coke oven emissions are correlated with mortality from various types of cancer, further demonstrating the additivity of time and concentration.

Overall mortality of white coke oven workers is somewhat less than expected. When mortality is categorized by cause, the rates for coke oven workers are significantly elevated for malignant neoplasms (relative risk, RR 1.34; $p<0.01$), for malignant neoplasms associated primarily with respiratory cancer (RR 2.85; $p<0.01$), for kidney cancer (RR 7.49; $p<0.01$), and for prostate cancer (RR 1.64; not significant).

The study showed that men employed at full-time topside jobs for five years or more have a relative risk of lung cancer of 6.87 ($p<0.01$), compared to risks of 3.22 ($p<0.01$) for men with five years of mixed topside and side-oven experience and 2.10 ($p<0.05$) for men with more than five years of side-oven experience. These data indicate a definite gradient in response based on both type and duration of exposure.

Overall, the study confirmed the Lloyd[445] findings of a more than twofold excess of mortality due to respiratory cancers in all coke oven workers. A new finding of Redmond's was a significant excess of kidney cancer among coke oven workers (RR 7.49; $p<0.01$).

Redmond concludes that the 6.87 ($p<0.01$) relative risk for malignant neoplasms of the respiratory system for men employed full-time topside, and the 1.70 relative risk (not significant) for men employed less than five years, constituted further evidence of a dose-response relationship.

In a later paper, Redmond[450] summarized and analyzed additional data (from 1967 to 1970) for the cohort that demonstrated a consistent increase in the level of risk of malignant neoplasms of various sites with increased exposure for each of the coke oven exposure groups studied. Further, the risk for side-oven workers, which had not been statistically significant in the earlier studies, reached significance (RR 1.79; $p<0.05$). Although no dose-response relationship was apparent, the relative risk for cancer of the pancreas and the relative risks for respiratory diseases other than cancer increased markedly with length of exposure (see Table 6-45).

A recent study by Redmond et al.[451] again confirmed elevated risks for coke oven workers for lung cancer (44 deaths versus 24.5 expected; RR 2.01; $p<0.01$), and genitourinary cancer (RR 1.82; $p<0.05$), due primarily to a fivefold increase in kidney cancer. Data on non-oven coke plant workers continue to demonstrate excess kidney cancer, and the most recent studies in this series show that incidences of buccal and pharyngeal malignancies are highly significant. The overall conclusion of the paper is that "these observations indicate the need to consider non-oven as well as oven workers when evaluating cancer hazards in the coke plant." This finding is of great importance since non-oven coke plant workers are exposed to considerably lower concentrations of coke oven emissions and, hence, of POM than oven workers.

Hendricks et al.[452] examined the incidence of cancer of the scrotum among 82 wax pressmen over a 20-year period. The refinery population as a whole had a cancer incidence close to that expected on the basis of the age-adjusted rate for the general white male population. The 82 wax pressmen, with 10 or more years of employment, represented 3 percent of the refinery population but accounted for about 11 percent of the cancer cases. The cancer rate among wax pressmen was 4 times that of refinery workers as a whole, which was similar to that of the general male population. Of the 19 cancer cases among pressmen with 10 years or more experience, 11 were scrotal cancers. The greatly increased risk for scrotal cancer of the pressmen can be accounted for by their skin contact with the crude wax, which contained large amounts of aromatic compounds. Workers handling the finished wax did not show a significantly elevated risk.

Hammond et al.[453] conducted a prospective study of mortality from 1960 through 1971 among 5,939 male hot pitch roofers and waterproofers. Motivation for the study was provided by the knowledge that most of these workers were frequently exposed to extremely high concentrations of BaP in the air they breathed. The principal criterion for inclusion of an individual in the cohort was that he must have been a member of the United Slate, Tile and Composition Roofers, Damp and Waterproof Workers' Association for at least nine years prior to the onset of follow-up on January 1, 1960. The inclusion of workers irrespective of whether active, probational or in retired status tended to reduce the healthy worker bias which would apply to the follow-up only of active workers. The quality of follow-up for mortality was excellent: only 151 men were unsuccessfully traced with respect to survival status during the 12 year follow-up period; 1,798 deaths were recorded in all.

The cohort person-years of experience were classified according to years of past membership in the union. Observed deaths in major cause categories were compared with expected numbers. The latter were obtained by applying age and calendar year specific rates for the entire male population of the United States to the corresponding person-years of exposure in the cohort classes.

In all age classes except 50-59 years, the ratios of observed to expected deaths slightly exceeded 1. Thus, if there was a healthy worker bias, it was more than exceeded by generally higher rates of death in the worker cohort than among the general population of United States males. The overall mortality ratio for workers with only 9-19 attained years since joining the union was 1.02, compared to 1.09 for those with 20 or more attained years since joining. The respective cancer mortality ratios of 1.07 and 1.45 showed the levels of these ratios were not generally greater than for certain other causes of death such as accidents (1.59 and 1.41, respectively) and deaths from other respiratory causes (1.96 and 1.67, respectively). The mortality ratios for all other causes than specified above, however, were less than 1. For cancer of the lung, the mortality ratio rose from 0.92 for 9-19 years of membership to 2.47 for 40 or more years. Taken in itself, the latter gradient might be dismissed as merely the result of an attenuation in the healthy worker bias with the passage of time. However, similar gradients were not seen for the mortality ratios in the other major cause of death categories. Thus, there was support for the concept that there was an increased hazard of lung cancer with extended duration of exposure.

As to whether excess mortality can be attributed to BaP exposures, the question is moot. This is because, even though there were extremely large BaP dose gradients (from averages near 14,000 ng/1000m^3 in the roof tarring areas to 6,000,000 ng/1000m^3 near kettles), internal dose-response comparisons were not presented in the article. Presumably the data were not available for different categories in the cohort of workers.

6.7.2 Community Studies

The observation of a link between an occupational chemical exposure and irreversible disease inevitably prompts a similar investigation in the community setting. With POM, investigators have long sought a measure of the excess risk of lung cancer presumably attributable to ambient air pollution by POM. None of these attempts have provided unequivocal results and thus a concensus has not yet been reached regarding a causal association between cancer and POM in air. Nevertheless, it is commonly assumed that the incidence of disease from exposure to a carcinogen is directly proportional to the concentration to which the population is exposed, regardless of how small the dose may be. The difficulty in evaluating the results of community studies involving low level carcinogen exposures is that the excess risk ratio relative to "background" cancers in the population is usually extremely low. However, a ratio of 1.05 or less would still represent a large number of cancer deaths per year. Several major attempts have recently been made to quantitate the carcinogenic risks of POM present in ambient air.[179,454,458]

A great problem in the analysis of community air pollution studies is the confounding effect of cigarette smoking which is the major etiologic agent in lung cancer.[434] A second confounding factor is occupational exposure. This is of much smaller magnitude for the general population than smoking, but it is thought to be large in comparison to any effect caused by air pollution.[433] Thus, an increase in lung cancer caused by air pollution will be difficult to attribute to a particular etiologic agent. Few of the community studies of air pollution have controlled for these two factors.

Hammond[459] has pointed out other problems that arise in air pollution studies. First, the kinds of information on which estimates of increased risk in smokers and certain occupational groups are based are not obtainable for the subjects in general air pollution studies. These include the interval since first exposure and a rough estimate of the degree of exposure. Second, the amount and type of air pollution varies within the same metropolitan area from day-to-day and from year-to-year. Also, the levels of air pollution in most cities have changed substantially over the past 30 years so that recent measurements do not give an accurate picture of exposure at an earlier time. Even current sampling procedures may not adequately reflect differences in exposure in different neighborhoods of the same metropolitan area. The high degree of mobility among Americans further complicates the problem of determining the type and degree of exposure.

Even if excess risk for certain diseases is attributable to air pollution, there is the problem of identifying the specific etiologic agent(s) in air pollution that may be responsible for an increase in certain diseases. As in the case of occupational exposure, community air pollution contains other agents besides POM and these may exert their own separate effects on health or may interact with POM. In some studies of community air pollution, BaP (a known animal carcinogen) is used as an indicator for the presence of other POM. However, BaP is an imperfect indicator because the proportion of BaP to other POM varies with the type of pollution (automobile exhaust, coal burning, etc.).[460] It has also been shown experimentally that BaP alone does not adequately account for the carcinogenicity of complex mixtures such as air pollution and cigarette tar.[461,462]

Several approaches have been taken in the conduct of large scale epidemiologic studies of the association between general levels of air pollution and cancer mortality. In general, four methodological categories can be designated: (1) urban-rural comparisons; (2) migrant studies; (3) regression analyses of lung cancer death rates for residents of countries or states with respect to gross indicators of cigarette consumption, industrialization, and air pollution; and (4) retrospective and prospective analyses of data for sampled persons with individually identified exposure and background characteristics.

6.7.2.1 Urban-rural comparisons--The urban-rural studies (in which pollutant levels are not measured) were conducted by: Mancuso[463,464] in Ohio; Griswald[466] in Connecticut; Haenszel and coworkers[467,468] in Iowa; in the United States by Hoffman and Gilliam,[469] Manos and Fisher,[470] and Prindle[471]; by Stocks[472] in the United Kingdom; and by Curwen and coworkers[473] in England and Wales. Without exception, a high to low urban-rural gradient in overall age-adjusted death rates was observed. For lung cancer in particular, the ratio of age-adjusted mortality rates for males in urban versus rural locations was consistently in the neighborhood of 2:1 (Tables 6-46 and 6-47). While there is general agreement on the existence of an "urban factor" for cancer mortality, there is no consensus that the "urban factor" is air pollution. Alternative explanations proposed to account for the urban-rural differences in lung cancer mortality include: (1) migration to cities for medical treatment; (2) increased bacterial and viral infection in cities; (3) increased bronchitis in cities; (4) increased occupational exposure to carcinogens; and (5) differences in smoking habits.

However, studies of local variations by Winklestein and coworkers[474] in Buffalo and Hagstrom and coworkers[475] in Nashville showed no consistent relationship between air pollution and lung cancer incidence within the urban environment. These studies tend to support the contention that not all of the urban-rural gradient should be attributed to the effects of air pollution. An additional finding in both studies, which could not be explained, was an association between stomach cancer and particulate air pollution. The possibility that particulates were cleared from the lungs by mucociliary action and subsequently swallowed seems obvious. However, the possible contribution of dietary factors was not considered in these or any other studies of POM pollution and human cancer. In addition, fundamental differences in urban-rural dietary habits may be confounding any interpretation of cancer mortality data with regard to air pollution in general and POM pollution in particular. The quantitative importance of dietary sources of POM relative to that which is inhaled necessitates a more careful consideration of this parameter in future epidemiologic research.

Therefore, after adjusting for age and cigarette smoking habits, there seems to be an unwarranted tendency to equate the residual urban-rural gradient

Table 6-46. AVERAGE ANNUAL AGE, RACE, AND SEX SPECIFIC MORTALITY RATES PER 100,000 POPULATION FOR CANCER OF THE LUNG IN THE UNITED STATES, 1948-49[a] [469]

Age (years)	Total				
	White		Nonwhite		
	Male	Female	Male	Female	All persons
Total crude rate	185.6	46.0	103.7	26.3	110.1
Total adjusted rate[c]	183.1	43.6	130.6	34.1	110.1
0-29	2.3	1.5	3.1	0.9	1.9
30-34	14.3	6.7	25.7	7.3	11.0
35-39	41.4	14.4	45.4	19.2	28.1
40-44	93.7	25.1	114.8	41.6	61.0
45-49	229.5	50.4	211.8	63.8	139.6
50-54	427.0	73.7	397.3	87.9	249.3
55-59	679.7	114.0	558.8	107.6	392.9
60-64	861.3	161.7	578.1	118.4	501.3
65-69	881.9	212.9	439.1	120.5	515.3
70-74	874.6	260.2	320.3	135.0	530.5
75 and over	698.0	297.8	372.2	124.5	462.9
0-44	16.4	5.7	18.4	6.6	11.2
45-64	517.6	94.2	392.3	88.0	300.6
65 and over	824.1	254.0	389.3	125.2	503.1

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[a] Deaths according to International List Nos. 47b, c, d, and e (1948) and Nos. 162 and 163 (1949); population according to 1950 census.

[b] Urban population is generally that living in places of 2,500 and over.

[c] Adjusted to the age distribution of the total United States population at the 1950 census.

Table 6-47. AGE-ADJUSTED[a] CANCER-INCIDENCE RATES PER 100,000 POPULATION, BY PRIMARY SITE AND SEX FOR URBAN[b,c] AND RURAL AREAS: NEW YORK STATE (EXCLUSIVE OF NEW YORK CITY), 1949-51; CONNECTICUT, 1947-51; AND IOWA, 1970[465]

Primary site	Urban			Rural			Ratio: Urban/Rural		
	New York	Connecticut	Iowa	New York	Connecticut	Iowa	New York	Connecticut	Iowa
				MALES					
All sites	264.5	271.8	350.6	212.8	200.3	250.2	1.24	1.36	1.40
Buccal cavity and pharynx	15.8	17.9	32.1	12.9	13.5	24.1	1.22	1.33	1.33
Lip	6.8	5.4	17.3	3.8	5.6	15.7	1.79	0.96	1.10
Digestive system	87.1	98.3	87.6	68.6	69.1	68.7	1.27	1.42	1.28
Esophagus	6.6	8.7	3.9	3.7	3.7	2.1	1.78	2.35	1.86
Stomach	23.7	28.5	24.7	20.8	21.3	24.7	1.14	1.34	1.00
Intestines	23.9	25.6	26.9	17.9	17.5	18.6	1.34	1.46	1.45
Rectum	18.3	20.1	16.3	13.8	15.5	12.4	1.33	1.30	1.31
Other digestive organs	14.6	15.4	15.8	12.4	11.1	10.9	1.18	1.39	1.45
Respiratory system	37.9	35.2	32.8	26.5	20.5	12.1	1.43	1.72	2.71
Lung and bronchus, total	27.2	28.0	29.0	19.3	17.8	10.2	1.41	1.57	2.84
Primary	16.3			9.3					
Unspecified	10.9			10.0					
Larynx	5.4	5.8	2.9	3.7	1.4	1.4	1.46	4.14	2.07
Male genital organs	32.5	33.2	43.5	27.7	27.0	39.0	1.17	1.23	1.12
Prostate	28.7	27.8	37.8	24.1	23.3	34.2	1.19	1.19	1.11
Urinary organs	18.2	19.4	25.1	13.8	15.0	16.5	1.32	1.29	1.52
Kidney	4.6	5.6	4.8	3.5	4.1	3.5	1.31	1.37	1.37
Bladder and other	13.6	13.8	20.3	10.3	11.0	13.0	1.32	1.25	1.56
Skin	34.7	31.0	72.1	32.6	30.2	52.1	1.06	1.03	1.38
Lymphatic tissues	9.9	7.9	12.2	8.0	5.1	6.1	1.24	1.55	2.00
Leukemia	8.9	9.9	d	6.8	7.5	d	1.31	1.32	---

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[a] Rates adjusted for age to the total population of the United States, 1950.

[b] For New York State and Iowa, "urban" includes all incorporated places of 2,500 population and over, and certain other political subdivisions so classified by the U.S. Bureau of the Census under special rules relating to population size and density; for Connecticut, "urban" comprises 76 towns with high population density.

[c] Rates for urban and rural areas have not been adjusted to correct for bias in reporting place of residence on medical records.

[d] Comparable Iowa data not available.

[e] In Iowa, unspecified uterus was distributed between the cervix and corpus, based on additional data collected for a sample of cases and on histologic-type information.

Table 6-47 (continued). AGE-ADJUSTED[a] CANCER-INCIDENCE RATES PER 100,000 POPULATION, BY PRIMARY SITE AND SEX FOR URBAN[b,c] AND RURAL AREAS: NEW YORK STATE (EXCLUSIVE OF NEW YORK CITY), 1949-51; CONNECTICUT, 1947-51; AND IOWA, 1970[465]

Primary site	Urban			Rural			Ratio: Urban/Rural		
	New York	Connecticut	Iowa	New York	Connecticut	Iowa	New York	Connecticut	Iowa
				FEMALES					
All sites	248.2	261.1	351.6	218.3	204.4	261.5	1.14	1.28	1.34
Buccal cavity and pharynx	3.8	3.1	7.2	2.9	2.6	4.1	1.31	1.19	1.76
Lip	0.4	0.2	1.2	0.3	0.8	0.8	1.33	0.25	1.50
Digestive system	62.9	73.6	68.5	55.6	59.0	65.2	1.13	1.25	1.05
Esophagus	1.1	1.5	0.6	1.2	0.7	1.2	0.92	2.14	0.50
Stomach	11.7	15.5	13.1	10.1	12.0	14.1	1.16	1.29	0.93
Intestines	25.7	28.4	29.3	22.3	22.2	23.8	1.15	1.28	1.23
Rectum	11.9	14.0	10.0	9.9	13.6	11.0	1.20	1.03	0.91
Other digestive organs	12.5	14.2	15.5	12.1	10.5	15.1	1.03	1.35	1.03
Respiratory system	5.1	5.8	9.0	4.2	4.2	6.3	1.21	1.38	1.43
Lung and bronchus, total	3.2	4.7	7.8	3.0	3.8	5.3	1.07	1.24	1.47
Primary	1.3			1.2					
Unspecified	1.9			1.8					
Larynx	0.3	0.4	0.9	0.3	0.3	0.0	1.00	1.33	---
Breast	57.4	59.8	78.0	49.9	49.4	62.4	1.15	1.21	1.25
Female genital organs	58.1	58.4	88.7	53.2	43.4	59.8	1.09	1.35	1.48
Cervix	26.5	22.1	46.8[e]	23.4	15.0	26.4[e]	1.13	1.47	1.77
Corpus	15.8	12.0	21.2[e]	14.9	8.3	17.3[e]	1.06	1.45	1.23
Uterus, unspecified	1.9	8.9	---	2.1	7.2	---			
Ovary	9.5	13.1	15.1	9.2	11.4	12.3	1.03	1.15	1.23
Urinary organs	7.3	7.7	10.6	5.6	6.3	6.1	1.30	1.22	1.74
Kidney	2.8	3.6	3.3	1.9	2.9	2.8	1.47	1.24	1.18
Bladder and other	4.5	4.0	7.3	3.7	3.3	3.3	1.22	1.21	2.21
Skin	23.9	22.0	45.4	20.9	18.5	24.9	1.14	1.19	1.82
Lymphatic tissues	6.9	5.6	7.2	6.1	4.2	5.5	1.13	1.33	1.31
Leukemia	5.8	7.8	d	5.3	5.2	d	1.09	1.50	---

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[a] Rates adjusted for age to the total population of the United States, 1950.

[b] For New York State and Iowa, "urban" includes all incorporated places of 2,500 population and over, and certain other political subdivisions so classified by the U.S. Bureau of the Census under special rules relating to population size and density; for Connecticut, "urban" comprises 76 towns with high population density.

[c] Rates for urban and rural areas have not been adjusted to correct for bias in reporting place of residence on medical records.

[d] Comparable Iowa data not available.

[e] In Iowa, unspecified uterus was distributed between the cervix and corpus, based on additional data collected for a sample of cases and on histologic-type information.

in cancer death rates with the direct effects of inhaled air pollutants. The studies by Winklestein et al.[474] and Hagstrom et al.[475] should help to inhibit that tendency. The major question which remains unanswered is what part of the urban-rural gradient can be attributed to the air pollution gradient to which it corresponds.

6.7.2.2 Migrant studies--Studies on lung cancer rates in migrant populations (in the absence of pollution monitoring data) tend to indicate that exposures occurring early in life can lead to the development of cancers even though the victims have migrated to a different environment. In this regard, it can be seen that the age-standardized lung cancer death rates among British and Norwegian migrants to the United States are intermediate between those in their original homeland and adopted country.[476] Other studies of cancer mortality among migrants support the concept that these persons are affected by their former environments and length of exposure in that environment.[463,477-480] It is suggested that differences in smoking habits are unlikely to account for discrepancies in lung cancer rates among migrant and resident populations.

6.7.2.3 Regression analyses--On the assumption that populations exposed to POM from the petroleum industry could be expected to have higher rates for certain cancers than unexposed populations, Blot et al.[435] conducted a survey of cancer mortality from 1950 to 1969 in 39 counties where the petroleum industry is most heavily concentrated. They found that white male residents of these counties had significantly higher rates for cancer of the lung, the nasal cavity and sinuses, and the stomach compared with the residents of counties with similar demographic features but without petroleum-processing plants. However, these correlations do not necessarily establish cause-and-effect relationships. Ratios of age-adjusted mortality rates among white males in the petroleum-industry counties to the rates in control counties were: 1.15 for lung cancer (significant at 1 percent level); 1.48 for cancer of nasal cavity and sinuses (significant at 1 percent level); 1.09 for stomach cancer (significant at 1 percent level). The ratio for all sites combined was 1.06 (significant at the 1 percent level). White females in petroleum-industry counties also had significantly elevated rates for lung cancer (though not for cancer of the nasal cavity and skin, nor for all cancers combined), suggesting the possibility that community exposure might be involved as well as occupational exposure. Data from Blot et al.[435] are given in Table 6-48.

Using death certificate data from 1968 to 1969, Menck et al.[481] found elevated lung cancer mortality rates in white males living in certain heavily industrialized areas of Los Angeles County. Populations at risk were determined from the 1970 Census data. Age-adjusted lung cancer mortality rates for Caucasian males and females were calculated for 13 areas from Los Angeles County death certificates. A direct method of age-adjustment using the U.S. 1970 Census population as a standard was carried out, and socioeconomic class index based on average income and education level was assigned to each census tract.

The age-adjusted lung cancer rates in 1968 and 1969 for male Caucasians in the 13 study areas ranged from 43 to 75 per 100,000. In three contiguous areas of south-central Los Angeles County, however, the mortality rate was 70 per 100,000 or greater. The excess of male lung cancers for these areas was 40 percent above the rate for the rest of Los Angeles County.

Table 6-48. RATIOS OF AGE-ADJUSTED MORTALITY RATES, 1950 TO 1969, AMONG WHITE MALES IN PETROLEUM INDUSTRY COUNTIES TO THOSE IN CONTROL COUNTIES BY CANCER SITE[435]

Cancer site	Rate
Buccal cavity and pharynx	1.04
Esophagus	1.06
Stomach	1.09[a]
Colon	1.02
Rectum	1.07[b]
Liver	1.06
Pancreas	1.05
Nasal cavity and sinuses	1.48[a]
Larynx	1.09
Lung	1.15[a]
Prostate	0.98
Testis	1.10[b]
Kidney	1.05
Bladder	1.02
Melanoma and other skin	1.10[b]
Brain	0.94[b]
Thyroid and endocrine	1.04
Bone and connective tissue	0.98
Hodgkins's disease	0.96
Other lymphomas	1.01
Multiple mycloma	1.05
Leukemia	1.03
All sites combined	1.06[a]

[a] $P<.01$.

[b] $.01<P<.05$.

The inhabitants of the three areas of south-central Los Angeles County are mainly lower and middle class, and the lung cancer rates for Los Angeles County were 1.73 times higher in the lower than in the upper socioeconomic classes. However, the excess for the three south-central areas remained when deaths for the 13 study areas were examined for the lower socioeconomic groupings.

Measurements of BaP and other PAH in the air and soil (made at four sampling stations with and adjoining the three south-central areas) showed the highest pollution levels at the center of the three study areas with increased lung cancer mortality.

Neither smoking nor occupational history data were available in this study, but the authors argue that neither smoking nor occupational exposure could account for the excess. They suggest that synergistic action between smoking and neighborhood air pollution, primarily of industrial origin, provides the best explanation of the elevated lung cancer rates in south-central Los Angeles County.

A follow-up study[482] including additional mortality data from 1970 and morbidity data from 1972 confirmed the findings of an elevated lung cancer rate for south-central Los Angeles County. Age-adjusted lung cancer rates per 100,000 were 70.9, 70.2, and 69.2 for the three south-central areas compared to an average rate of 55.8 for all 14 study areas in Los Angeles County. Henderson et al.[482] reported that the increased risk was present in different social classes as well as in six of eight non-factory occupational categories. Females did not have an elevated risk. Although current occupation was an important factor in the lung cancer risk of Los Angeles County men, current occupation did not explain the excess male risk in the south-central area. The authors noted that detailed work histories might reveal an occupational risk that could account for the observed increased cancer risk. It was suggested that the excess lung cancer rate was not due to differences in smoking, since cancers of other sites normally associated with smoking showed no excess in the three south-central areas.

In a study of respiratory cancer in ten Polish cities, an apparent correlation was found between cancer death rates and degree of pollution by representative POM.[483] Data on ambient levels of dust, tars, BaP, BeP, BghiPer, and pyrene for the period 1966 to 1967 were compared with the age- and sex-adjusted respiratory cancer death rates for the period 1962 to 1963 in the same cities (Table 6-49). For the most part, it was shown that cities with the highest death rates had the highest levels of POM pollution (correlation coefficient, R = 0.66). No apparent relationship was seen between respiratory cancer death rate and air pollution as measured by other indices (e.g., dust, tar substances). Caution must be exercised, however, in the interpretation of cancer incidence data in the absence of historical information on ambient levels of POM. The long latency period for tumor appearance (10 to 30 years) often makes it impossible to establish levels of exposure at the time of the initial carcinogenic event. Moreover, it cannot be stated with certainty whether carcinogenesis in humans is determined by intensity or duration of exposure, or a combination of both. In addition, a presumed lognormal distribution of incubation periods for neoplastic disease may complicate the attempt to correlate time of exposure with carcinogenic effect.[484]

Stocks has carried out many studies relating lung cancer mortality to air pollution. In 1952,[485] he reported that, in towns, lung cancer mortality

Table 6-49. RANKING OF THE CITIES INVESTIGATED BY THE VALUES OF THE ATMOSPHERIC AIR POLLUTION INDICES AND DEATH RATE IN THE POPULATION FROM CANCER OF THE LUNGS AND BRONCHI (THE NUMBERS IN PARENTHESES INDICATE THE RANK OR SEQUENTIAL POSITION OF THE CITY BY THE GIVEN CHARACTERISTIC)[483]

City	Average concentration in the atmosphere in the period 1966-1967						Standardized combined death rate for the years 1962 and 1963
	Dust in μg/m^3	Tar substances in μg/m^3	BaP	BeP	BghiPer	P	
			In μg/1000 m^3				
Gdansk	121 (1)	21 (1)	74 (7)	37 (7)	79 (7)	107 (8)	31.9 (7)
Katowice	293 (8)	33 (7)	75 (8)	39 (8)	81 (8)	95 (7)	31.3 (6)
Kratow	248 (7)	34 (8)	63 (6)	32 (6)	69 (6)	90 (6)	28.4 (5)
Lodz	155 (3)	28 (5)	45 (2)	22 (2)	46 (2)	54 (2)	27.1 (3)
Poznan	155 (4)	23 (3)	49 (3)	26 (3)	53 (3)	55 (3)	27.7 (4)
Szczccin	175 (5)	26 (4)	53 (4)	27 (4)	57 (4)	61 (4)	33.1 (8)
Warszawa	133 (2)	21 (2)	29 (1)	15 (1)	32 (1)	35 (1)	16.8 (2)
Wroclaw	191 (6)	30 (6)	61 (5)	29 (5)	68 (5)	79 (5)	13.8 (1)
Zabrze	336 (9)	38 (9)	117 (9)	65 (9)	117 (9)	146 (9)	47.3 (9)

increased in proportion to the number of inhabited dwellings (from a comparative mortality ratio of 89 in towns with less than 20,000 occupied dwellings to 162 in towns with over 200,000 occupied dwellings). Differences in smoking habits could not account for the lung cancer excess in towns compared with rural areas. Stocks put forward the hypothesis that the effects of tobacco and atmospheric pollution are additive.

Stocks and Campbell[472] compared the lung cancer rates among men with different smoking habits in a rural area, a mixed urban-rural area, and a highly urbanized area (Liverpool County borough) using data from a study of environmental histories of persons with and without cancer carried out by the British Cancer Campaign. The death rates were related to levels of BaP and other POM and sulfur dioxide in the air in each area.

The rural death rate increased with increased number of cigarettes smoked per week. Liverpool rates were higher than the rural rates in every smoking category, but the urban-rural ratio decreased progressively from about 9 to 1 for nonsmokers to near unity for heavy cigarette smokers.

There was an "absolute urban excess" in each smoking group, suggesting that an urban factor was added to the effects of smoking. Stocks and Campbell estimated that about half the male lung cancer deaths in Liverpool were due to smoking and about three-eighths were due to "a factor which is only slightly present in the rural areas."

The level of air pollution increased with increasing urbanization. The concentration of BaP in Liverpool was from 8 to 11 times that in rural areas, and this ratio corresponded to the estimated mortality ratio among nonsmokers in urban and rural areas.

Stocks[486,487] reported significant correlations between SMR's for lung cancer and bronchitis and air pollution (undissolved deposit and smoke) levels in 58 county boroughs (towns) in England and Wales when population density was held constant. Stomach cancer was also positively correlated with air pollution. Lung cancer gave a correlation of 0.500 with the amount of deposit when the population density was held constant ($p<.002$). Lung cancer gave a significant partial coefficient with smoke of 0.510 ($p<.01$). Bronchitis gave a partial coefficient with amount of deposit of 0.579 in males and 0.511 in females.

In an expanded study,[488] a high correlation was reported between smoke density and lung cancer mortality ($r = 0.873$). Differences in mortality were only partially explainable by social differences (housing indices based on numbers of persons per room and the proportion of employed and retired males in unskilled jobs). Bronchitis and pneumonia in males ($r = 0.869$ and $r = 0.666$, respectively) and bronchitis in females ($r = 0.751$, significant at the 5 percent level) were also strongly related to smoke. Analysis for the relative correlation of four different POM indicated that BaP was the "substance of prime importance" for lung cancer. The trace elements beryllium and molybdenum also showed associations with lung cancer.

Stocks[489] reported the results of three separate analyses:

(1) Lung cancer mortality showed "substantial and independent correlations" with smoking and air pollution (measured by smoke, BaP, and three other POM) in six European cities and two areas of Wales.

(2) Lung cancer mortality showed positive correlations with the per capita consumption of cigarettes and solid fuel (but not with liquid fuel) in

19 countries. Smoking appeared to account for about two-thirds of the deaths in the average country and coal consumption for about one-third.

(3) There was a large urban excess of lung cancer in English metropolitan areas relative to surrounding regions after the effects of differences in social and other factors were eliminated. This excess was attributed to air pollution.

The analysis of lung cancer and mortality in relation to smoking and per capita fuel consumption in 19 countries was repeated[490] with more recent mortality data. When lung cancer rates were adjusted for smoking differences, the adjusted rates were generally higher in countries with high coal consumption than in countries with low coal consumption. Smoking showed a greater effect than air pollution from solid fuel burning on the lung cancer rate in men aged 35 to 44 (the correlation coefficients with lung cancer were 0.634 for smoking and 0.470 for solid fuel), but in men aged 55 to 64, air pollution showed a greater effect even when smoking was held constant (the correlation coefficients were 0.599 for solid fuel and 0.380 for smoking).

Carnow and Meier[491] reviewed the multiple regression analysis performed by Stocks and Campbell[489] on the age and sex-specific and adjusted lung cancer death rates for 19 countries. After taking account of the influence of the covariate defined as per capita cigarette consumption, the analysis indicated a substantial residual association between the age-sex adjusted lung cancer death rate and per capita fuel consumption as an index of air pollution; the average age-sex adjusted lung cancer death rates were estimated to increase by a factor of about 20 percent per metric ton of solid fuel consumed per capita per year. However, since fuel consumption may be a surrogate for numerous industrial, climatic, and other national characteristics, it would be presumptuous to attribute all of this relationship to the effects of air pollution.

In a similar regression analysis of age-sex-race specific and adjusted lung cancer death rates for persons 35 years of age and older in the 48 contiguous United States, cigarette sales per person and average BaP concentrations in the ambient air were employed as the regressors. Population-weighted averages of BaP concentrations in samples taken from 1967 to 1969 in urban and non-urban strata throughout the country were used by the authors as a convenient representation of general air pollution levels. The specific BaP measure was used as an "alias" or surrogate variable to represent all forms of general air pollution in the states. Thus, a unit of "air pollution" was defined as a one-year average of 1 μg BaP/1000m^3. It was found that the lung cancer death rates for white males increased by a factor of 5 percent for each such unit of pollution; the increase was 15 percent for non-white males, 6 percent for white females, and 1 percent for non-white females.

The authors felt that the foregoing effects could have been overestimates, because 1967-69 levels of BaP were only about 40 percent of their general levels in the more typical period of the early 1950's. It is questionable, however, whether secular changes in BaP adequately reflect long-term changes in overall air pollution levels. Then, if BaP is an inadequate surrogate for long term changes, perhaps downward adjustment by a factor of 40 percent would be an over-correction. This analysis by Carnow and Meier[491] was included in the National Academy of Science's report on Biologic Effects of Atmospheric Pollutants: Particulate Polycyclic Organic Matter.[179]

6.7.2.4 Retrospective and prospective analyses--This category of community studies on POM includes both prospective and retrospective large scale sampling designs. Prospective studies of lung cancer death rates were carried out by Buell and Dunn[492] in male veteran residents of California and by Hammond and Horn[493] in United States white male veterans. Retrospective studies were performed by Dean[494] in Northern Ireland, Haenszel et al.[467, 468] in the United States, and by Hitosugi[495] near Osaka. The designs of these studies permitted finer adjustment for the individually determined background characteristics of age, sex, and smoking habits. In some of the designs, individual and family histories permitted the evaluation of the duration of urban or rural residence and other factors in conjunction with the urban-rural exposures. The characteristic high-to-low gradient in urban versus rural residence was consistently confirmed in all of these studies. When expressed per unit of BaP as an index of pollution, male lung cancer death rates increased by a factor of 4 to 5 percent per $\mu g/1000m^3$. These agreed well with the gradients estimated by the cruder population and regression studies previously cited.

Carnow and Meier's[491] proposal of a 1 μg BaP/$1000m^3$ "unit of pollution" may have been more for analytical convenience than as an index of widespread practical use. In addition to BaP concentrations, Hitosugi felt it necessary to characterize air pollution levels in terms of measured rates of dust fall, rates of sulfur dioxide accumulation, and suspended particulate concentrations. Carnow and Meier[491] have also pointed out that long term trends in predominant types of fuel might vitiate the validity of BaP as an index. Specifically, data from Finland and New Orleans did not conform well with the BaP index, primarily, it was believed, because of atypical fuel usage in those areas.

Even where the BaP index has seemed to perform well in past studies, the habitual use of a single component as an index and its possible incorporation into the language of regulations would seem to invite misinterpretation and improper manipulation. A more prudent approach would involve a multiple component index based on such measures as obtained in the study by Hitosugi.[495] It seems that Carnow and Meier would also prefer using a composite index, since one of their strongest recommendations was to obtain more precise measures of POM, particulates, and irritant gases in ambient air sampling. They also strongly recommended better documentation of cigarette smoking habits and obtaining a greater variety of samples from occupational and residential strata.

Taking a different approach based on the risk of highly exposed workers, Pike et al.[434] calculated a small but non-negligible risk associated with air pollution. Pike et al. used the level of exposure to BaP among British gas workers showing an excess lung cancer rate in order to extrapolate to the lung cancer rate attributable to BaP in general urban air pollution. They explain the algorithm used in the extrapolation as follows:

> "...The carbonization workers were exposed to an estimated 2,000 ng/m^3 BaP for about 22 percent of the year (assuming a 40-hour working week, 2 weeks paid leave, 1 week sick leave); very roughly, the men were exposed to the equivalent of 440 (2000 x 0.22) ng/m^3 BaP general air pollution. This exposure caused an extra 160/10^5 lung cancer cases, so that we may estimate, assuming a proportional effect, that each ng/m^3 BaP causes 0.4/10^5 (160/10^5 ÷ 440) extra lung cancer cases per year..."

This calculation yielded an excess rate of 18/100,000 lung cancer cases per year for a city with 50 ng/m^3 BaP air pollution. The authors took this calculation and Stocks's comparable findings as support for the "notion of a simple proportional relationship between increasing BaP concentration in the air and excess rate of lung cancer." Pike et al. further calculated that the excess lung cancer rate associated with smoking one cigarette per day (in the U.S.) was equivalent to the rate due to exposure to 10 ng/m^3 BaP in the air.

While occupational studies have provided strong evidence that exposure to high levels of POM causes an increased risk for cancer of several sites and for nonmalignant respiratory disease, the results of general air pollution studies remain inconclusive. At present, there seems to be no absolute consensus that the differences between lung cancer rates in urban and rural areas are due to general air pollution. In his review of the epidemiology of lung cancer in the United States, Higgins[496] has written that "while an urban effect is undoubted, it is still not certain that it is due to carcinogenic pollutants in the air." Goldsmith and Friberg,[497] in their review of the literature on air pollution and lung cancer, conclude that the evidence does not support the hypothesis that "pollution *per se* is the urban factor," but they add that it is also not possible to reject the possibility.

Other reviewers have concluded that while air pollution may be a significant factor in the etiology of lung cancer,[455-458] estimates of the magnitude of risk vary tremendously. At the International Symposium on General Air Pollution and Human Health with Special Reference to Long-Term Effects held in Stockholm, March 8-11, 1977 several conclusions were reached concerning carcinogenic effects of POM. Participants at the Symposium agreed on the following conclusions:[540]

1. Lung cancer is more common in urban versus rural areas.
2. Cigarette smoking is the major cause of lung cancer, and contributes to urban-rural differences.

3. Differences in lifestyle (e.g., alcohol consumption, nutrition) and occupation are not important causes of the urban-rural difference.
4. Combustion products of fossil fuels, probably acting together with cigarette smoke, can account for cases of lung cancer in large urban areas. The number of such cancers is in the order of 5 to 10 cases per 100,000 males per year.
5. Although well-documented urban-rural differences have been described for cancers in other organs, it is not possible to evaluate the role of air pollution in determining such differences.

When evaluating the carcinogenic threat of POM to man no one has yet considered the relevance of dietary sources or its impact on gradients of cancer for sites other than the respiratory tract. This may be especially important in light of the fact that the absolute rate of digestive tract cancer in both rural and urban populations can be considerably higher than the rate of respiratory tract cancer.[465,474,475] In addition, the oral administration of POM to experimental mammals commonly produces lymphomas, leukemia, and tumors of the lungs and endocrine tissues; thus the possibility is raised that the carcinogenic impact of POM in humans may be underestimated by considering only inhalation as the primary route of exposure, and lung cancer as the endpoint of greatest concern.

6.8 ECOLOGICAL EFFECTS

POM is ubiquitous in the natural environment, arising partly from human activity (e.g., petroleum spillage, transport of combustion products), but may also originate from natural sources, such as biosynthesis by plants, algae, and bacteria,[498,499] although some investigators have argued that aerobic and anaerobic bacteria do not synthesize POM.[500] Therefore, an assessment of the ecological impact of POM in our terrestrial and aquatic environments is complicated by uncertainty over the natural role which these compounds may have.

6.8.1 Effects on Microorganisms and Algae

Payer and coworkers[501] have indicated that the green alga, Scenedesmus acutus, may contain significant quantities of POM which vary depending on the geographical location where it is grown (Table 6-50). From the lognormal distribution of carcinogenic POM which was detected, these investigators concluded that most of the POM present was due to bioaccumulation from environmental (i.e., anthropogenic) sources as opposed to endogenous formation. Effects of these POM on algal growth were not indicated.

Although it was reported that phenanthroquinone, a degradation product of phenanthrene, reduced the survival of various algal species at concentrations of 40 to 800 µg/liter,[502] others demonstrated that POM at low levels can enhance algal growth.[503] The growth of Chlorella vulgaris, Scenedesmus obliquus, and Ankistiodesmus oranunii was enhanced by exposure to 10 to 20 µg/liter fluoranthene; 1,12-benzoperylene; 3,4-benzofluoranthene; indeno-(1,2,3,cd)pyrene; 1,2-benzanthracene; BaP; and DBahA. Moreover, the degree of growth enhancement appeared to correlate with carcinogenic potency.

Table 6-50. POLYCYCLICS CONTENT (ppb) OF DRIED MICROALGAE AS A FUNCTION OF GEOGRAPHICAL LOCATION[a] 501

	Dortland, Germany	No. of Samples	Bangkok, Thailand	No. of Samples
3,4-Benzopyrene	$39.5\ (2.149^{\pm 1})$	19	$1.39\ (1.605^{\pm 1})$	15
3,4-Benzofluoranthene	$85.4\ (1.795^{\pm 1})$	19	$5.94\ (1.639^{\pm 1})$	14
Indenopyrene	$50.9\ (1.923^{\pm 1})$	19	$2.36\ (1.516^{\pm 1})$	8
Fluoranthene	$444\ (1.505^{\pm 1})$	18	91.2 ± 38.2	8
1,12-Benzoperylene	$52.1\ (1.932^{\pm 1})$	19	3.89 ± 2.36	8
11,12-Benzofluoranthene	$36.3\ (1.863^{\pm 1})$	19	2.40 ± 1.02	8

[a] The scattering of values is indicated by median values and scattering factors in the case of lognormal distributions, or by arithmetic means and standard deviations in the case of normal distributions.

Following the demonstration that Chlorella vulgaris could utilize acetate in the growth medium to biosynthesize BaP, an hypothesis was presented that POM may act as an endogenous growth promoter in plants.[499] This contention is also supported by the observation that growth of higher plants (rye, radish, tobacco) is promoted by POM in the same fashion as the growth of algae.[503] However, in comparison to the POM accumulated in plants from anthropogenic sources, the contribution by endogenous synthesis is likely to be quite insignificant.[501,504]

In various microorganisms, POM may either promote or inhibit growth. Toxicity may be expressed more as a function of increasing concentration rather than specific chemical structure. On the other hand, Hass and Applegate[505] reported that a structure-activity correlation may exist for the effects of polycyclic aromatic hydrocarbons on the growth of Escherichia coli. At concentrations in the medium of 10^{-7} to 10^{-5} molar, anthracene, phenanthrene, chrysene, DBacA, and pentacene inhibited bacterial growth. The more angular configurations, 1,2-benzanthracene, DBahA, and BaP, promoted the growth of E. coli. It was concluded from these limited data that growth promotion may require the presence of POM with an angular acene conformation, whereas inhibition might occur with both linear and angular acene molecules.

Recent studies conducted with marine bacteria do not support the hypothesis that the effect of POM on microorganisms might be structure-specific. Instead, Calder and Lader[506] reported that aromatic hydrocarbons inhibited the growth of Serratia marinoruba and Vibrio parahaemolyticus in a manner which was dose-related and a function of water solubility. Thus, a saturated solution of BaP would have the same impact on bacterial growth as a saturated solution of naphthalene, even though their respective solubilities differ by several orders of magnitude. Consequently, when evaluating the effects of POM on aquatic organisms it may be necessary to balance inherent toxicity on a molar basis against water solubility. The significance of considering both variables in the ranking of POM for toxicity to marine bacteria is depicted in Table 6-51. Furthermore, one must recognize that under normal conditions the low-to-medium molecular weight POM may contribute less to overall environmental toxicity than the higher weight molecules because the former are more readily lost by volatilization and degradation.[506]

Poglazova and Meisel[507] reported that a wide variety of bacterial types can accumulate BaP without metabolizing it. Localization of BaP occurs in lipid granules and lipoprotein membranes, as would be expected based upon the lipophilicity of most POM. Other investigators[508] have reported that cultures of Pseudomonas aeruginosa and E. coli absorbed 90 percent of the BaP added to the culture medium, 10-26 percent of which was metabolized. Whereas these authors reported a growth-stimulating effect of BaP on bacteria, others[509] observed a BaP-induced disorganization of colony structure. The localization of POM in bacterial membranes is the most likely explanation for observations that BaP depressed the metabolism of lipids and inhibited the formation of the electron transport system in Staphylococcus aureus.[510,511] Whereas BaP was inhibitory to membrane formation and function, benzo[e]pyrene was not.

Several studies conducted with cultures of yeast have confirmed that BaP readily penetrates the cell, is partially metabolized, and becomes

Table 6-51. EFFECT OF AROMATIC HYDROCARBONS IN MARINE BACTERIA [506]

Hydrocarbon	Mol Wt	(A) Solubility[a] M	(B) Δ%/µmole[b]	(C) Toxicity Index (C = A x B x 10^6)
Naphthalene	128.16	2.43×10^{-4}	0.4	97
2-Methylnaphthalene	142.19	1.73×10^{-4}	2.1	363
2,6-Dimethylnaphthalene	156.22	0.83×10^{-5}	12.8	106
Phenanthrene	178.22	0.66×10^{-5}	4.0	26
Pyrene	202.24	0.73×10^{-6}	170	124
Benzopyrene	252.3	$\sim 0.30 \times 10^{-7}$	881	>26
2,3-Dihydroxynaphthalene	160.16	$>16.5 \times 10^{-4}$	.68	>1,120

[a] In distilled water at 25°C, 1 atm.

[b] Magnitude of the toxicity per µmole expressed as percent decrease in number of bacteria relative to growth of controls.

localized in lipid components of the cell, including liproprotein membranes.[512-514] As a result, qualitative and quantitative alterations in the formation of lipids, especially phospholipids, have been observed when Saccharomyces cerevisiae was exposed to BaP or DBahA. On the other hand, weak- or non-carcinogenic POM (e.g., pyrene, dibenz[a,c]anthracene) were reportedly without effect.

It has also been suggested that once polycyclic aromatic hydrocarbons are absorbed by microorganisms they can be transferred throughout the cell population by cell surface contact. Studies with various yeasts grown in the presence of BaP demonstrated that greater than half of the hydrocarbon accumulated by yeast cells could be transferred to recipient yeast cells which were grown in a normal medium.[515]

Sludge microorganisms were adversely affected by a variety of carcinogenic POM.[516] An inhibition of oxygen uptake of varying degrees was obtained with different sludge microorganisms exposed to: DBahA; 7-methyl-1,2-benzanthracene; 1,2,4,5-dibenzopyrene; MCA; 2-nitrofluorene; 2-fluoreneamine; N-2-fluorenylacetamide; 7,9-dimethylbenz[c]acridine; 7,10-dimethylbenz[c]-acridine; dibenz[a,h]acridine; dibenz[a,j]acridine.

6.8.2 Effects on Aquatic Organisms and Amphibians

The toxicity of POM has not been extensively studied in fish, amphibians, or aquatic invertebrates. Nevertheless, it is known that marine fish and invertebrates can accumulate BaP from polluted waters.[504,517-520] Furthermore, freshwater and marine fish are capable of metabolizing a wide variety of hydrocarbons.

The induction of cytochrome P450-dependent MFO activity in fish and invertebrates is now receiving considerable attention with respect to bioactivation of carcinogenic POM.[518] Investigators postulate that the capacity of aquatic organisms to absorb and metabolize POM is important not only in determining carcinogenic risk for that species, but may also have implications for the transfer of POM and its activated metabolites through the food chain.

Lee and coworkers[517] showed that ^{14}C-naphthalene and ^{3}H-BaP were readily taken up through the gills of three marine fish--mudsucker, sculpin, and sand dab. BaP was metabolized in the liver and, within a few hours, metabolites were transferred to the gall bladder and excreted predominantly in the urine. The main product of BaP metabolism was 7,8-dihydro-7,8-dihydroxybenzopyrene, which indicated that the pattern of biotransformation was similar to that in the rat. With naphthalene, however, a greater tissue uptake was demonstrated than for BaP, and excretion of metabolites was principally via the feces. The rate of elimination of radioactivity derived from ^{14}C-naphthalene was more rapid than for ^{3}H-BaP. Overall, it was concluded that marine fish are able to dispose efficiently of certain nonhalogenated polycyclic aromatic chemicals.

The fact that sub-mammalian species can metabolize POM would lead one to speculate that they may also be able to form activated intermediates from these compounds. Indeed, it has been shown that tumors can be readily induced in fish and amphibians by exposure to chemicals which are known to be carcinogenic in mammals. In addition, Ahokas and coworkers[521] recently demonstrated that the oxidative metabolism of BaP by trout liver led to the formation of reactive intermediates which became covalently bound by protein. Furthermore, trout liver microsomal enzymes activated BaP, 2-aminofluorene, and 2-acetylaminofluorene to compounds which were potent frameshift mutagens in the Ames Salmonella assay system. In comparison to the Sprague-Dawley rat microsomes, trout liver microsomes metabolized BaP 15 to 30 times faster when the quantity of BaP metabolites produced in 15 minutes was expressed per nmole of cytochrome P-450 or per unit of cytochrome c reductase.[522] Hepatic levels of cytochrome P-450 are higher than in the rat, whereas cytochrome c reductase levels are lower. However, the Vmax is higher than for the rat and the Km is lower when BaP is used as a substrate.

Carcinogenicity testing of POM in lower organisms has been pursued for many years. For the most part, compounds which are carcinogenic in mammals also produce hyperplastic reactions or tumors in lower animals. Arffman and Christensen[523] summarized much of the early work performed with the newt, a salamander. These studies showed that an early proliferation of the epidermis accompanies the subcutaneous injection of tar, BaP, and MCA in the tail region of the newt. They confirmed these results by showing that an epithelial proliferation commonly occurred with the injection of BaP, MCA, or DBahA. The highest incidence of epithelial reaction was obtained with DBahA.

Following the demonstration in the clawed toad, Xenopus laevis, that implantation of MCA crystals induced lymphoid tumors, a similar study was undertaken to confirm this result using BaP.[524] The implantation of BaP crystals (1.5 mg) in the abdominal cavity of adult Xenopus laevis laevis produced lymphosarcomas in 11 of 13 animals within 86 to 288 days. Advanced tumors were found which affected the liver and kidneys and which were transplantable to immature recipient Xenopus.

POM are widely distributed in the environment as evidenced by their detection in sediments, soils, air, surface waters, and plant and animal tissues.[525] The ecological impact of these chemicals, however, is uncertain. Numerous studies show that despite their high lipid solubility, POM show little tendency for bioaccumulation in the fatty tissues of animals or man. This observation is not unexpected in light of convincing evidence to show that POM are rapidly and extensively metabolized. Since only low levels of POM are detected in plants and lower organisms,[525] transfer of POM through the food chain does not seem likely. The direct impact of POM on plants, animals, or the ecological balance of nature is difficult to evaluate, since few data are available which suggest that adverse effects may occur.

6.9 SUMMARY AND CONCLUSIONS

6.9.1 Absorption, Distribution, and Excretion

In the environment, exposure to POM occurs by direct inhalation of polluted air and tobacco smoke, by ingestion of contaminated food and water, and by dermal contact with soot, tars, and oils. Regardless of the route of exposure, it can be demonstrated in laboratory animals that POM are readily absorbed across all epithelia which are in contact with the external environment. The fact that POM are generally highly lipid-soluble neutral molecules greatly facilitates their passage through the predominantly lipid-like cell membranes of animals, including man.

Under environmental conditions, POM reach the lungs by adsorption on carrier particles. Moreover, the regional deposition and retention of inhaled POM in the respiratory tract will be primarily determined by the physical size of carrier particles and, to a lesser extent, by their composition. Once deposited in the lungs, two processes begin to act on the particulate material. First, depending on their size, particles are cleared from the respiratory tract by upward flow in the mucociliary tree or by phagocytic action of pulmonary macrophages. Second, adsorbed POM is eluted from the carrier particles and left free to react with the respiratory tissues or traverse the epithelium to reach the systemic circulation. A balance apparently exists between the rate of particle clearance and the degree of POM elution which ultimately establishes the degree of toxicant exposure via the lungs. However, clearance of particles by mucus and ciliary action does not necessarily remove them from the body, since most materials cleared from the lungs are subsequently swallowed, thus allowing for absorption via the gastrointestinal tract.

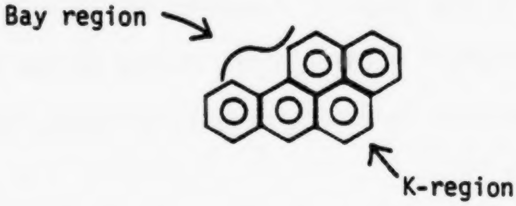
Upon reaching the bloodstream, POM are rapidly distributed to most internal body organs. Under experimental conditions with laboratory animals, the route of exposure has little apparent influence on the tissue localization of POM. Extensive localization in the fat and fatty tissues (e.g., breast) is observed, and suggests that these tissues may act as a chemical trap, creating a situation for sustained release of the unchanged substance. In pregnant rats, it is apparent that BaP and DMBA, but probably not MCA, are capable of transplacental passage and localization in the fetus.

Excretion of POM is rapid and occurs mainly via the feces; elimination in the bile may account for a significant percentage of administered doses. The influence of route of administration on patterns of POM excretion is not entirely clear. However, elimination of ^{3}H-BaP from various tissues (lung, liver, kidney, urine, feces) of the hamster following intratracheal instillation appeared to be biphasic. The slow phase of elimination from the lung (involving less than 1 percent of the treatment dose) seemed dependent on the administration of ^{3}H-BaP together with an adsorbent. It is not known to what extent bioexchange of the tritium label may have accounted for retention of radioactivity. Although some investigators find that retention of BaP in the lungs of experimental animals is dependent upon the co-administration of particulates, others maintain that carrier particles are more important in determining localization in the respiratory tract.

6.9.2 Metabolism and Metabolic Activation

The relative lack of chemical reactivity for tumorigenic POM has in the past been puzzling in light of their biological effects. More recently, however, it has become recognized that these molecules are enzymatically activated by oxidative mechanisms to form reactive electrophiles. These activated metabolites are capable of covalent interaction with cellular constituents (RNA, DNA, proteins), and it is suggested that one of these metabolites is the true ultimate carcinogenic form.

Metabolic reactions on the POM skeleton may take place at nearly any position, although chemical theory predicts that certain locations will be more reactive. The earliest theories designated a K-region having particular relevance to the biological activity of the molecule subsequent to oxidative attack. More recently, a "bay region" hypothesis was formulated which takes into account the ease of benzylic carbonium ion formation such as would be formed from the epoxides of diol-epoxides on tetrahydrobenzo rings in the "angular" region of a POM. These diol epoxides are postulated as ultimate carcinogenic metabolites of POM, a contention which is supported by circumstantial experimental data showing high mutagenicity/carcinogenicity for such structures and specific alkylation products of genetic material identical to that obtained with the parent compound under metabolic activation conditions. Examples of a K-region and a bay region on the BaP molecule can be depicted as follows:



POM is metabolized by the microsomal mixed-function oxidase system, often designated aryl hydrocarbon hydroxylase. This enzyme system is readily inducible and is found in most mammalian tissues, although predominantly in the liver. In conjunction with various P-450 type cytochromes, this enzyme complex is involved in detoxification of many xenobiotics, but may also catalyze the formation of reactive epoxide metabolites leading to carcinogenesis. A second microsomal enzyme, epoxide hydrase, converts epoxide metabolites of POM to vicinal glycols, a process which may also have critical importance to carcinogenesis.

Because of the importance of metabolic activation for the expression of carcinogenic effects by POM, the chemical fate of many representative compounds in mammalian cells has been extensively explored. By far the most widely studied of the POM has been BaP, one of the principal carcinogenic products from the combustion of organic material. The metabolites of BaP (and all POM) can be divided into a water-soluble and an organic solvent-soluble fraction. Components of the latter fraction are primarily ring-hydroxylated products, quinones, and labile epoxide intermediates. For BaP there are at least three dihydrodiols, three quinones, and two phenols which can be detected as positional isomers. The K-region (4,5-) and non K-region (7,8-; 9,10-) epoxides are precursors of the corresponding diols, which are formed by the action of the epoxide hydrase enzyme. A subsequent oxidative attack by the aryl hydrocarbon hydroxylase may convert the diols to diol epoxides, one of which (7,8-diol-9,10-epoxide) is an ultimate carcinogenic form of BaP.

In the water-soluble fraction containing BaP metabolites are mainly conjugates of hydroxylated products with glutathione, glucuronic acid, and sulfate. This group of metabolites is tenatively regarded to be composed of non-toxic excretion products.

The general scheme of metabolism for unsubstituted POM closely parallels that for BaP, although several other major environmental PAH and aza-arenes have not been studied. It is also evident that K-region derivatives of POM may be preferred targets for conjugation and excretion, whereas non-K-region epoxides undergo further reductions and oxidative attack to form toxicologically important molecules. For POM bearing alkyl substituents (e.g., DMBA, MCA), the primary metabolites formed are hydroxymethyl derivatives. Nevertheless, epoxidation reactions at K-region and non-K-region aromatic double bonds occur which are catalyzed by aryl hydrocarbon hydroxylase. Removal of activated intermediates occurs by conjugation with glutathione or glucuronic acid, or by further metabolism to tetrahydrotetrols.

Alternative explanations for the generation of reactive POM metabolites exist, although supporting evidence for their toxicologic significance is not as strong as for the diol epoxide (bay region) theory. One argument supports the role of reactive radical cation intermediates of POM as the critical metabolites capable for interaction with cellular constituents. Others have indicated that a 6-oxy-BaP free radical or a hydroxymethyl derivative of POM may be biologically important reactive metabolites.

The exact intracellular event by which a reactive POM metabolite initiates a toxic or carcinogenic response is not known. However, it is a widely held view that covalent binding of metabolites to DNA forms the molecular basis of the carcinogenic and/or mutagenic consequence of exposure to certain POM. In this regard it has been shown that carcinogenic POM in the presence of rat liver microsomes become bound to DNA and synthetic polynucleotides, and moreover that the extent of binding is correlated with the known carcinogenic potency of the compound and levels of microsomal enzyme activity. Covalent binding of reactive

POM metabolites also takes place with RNA and other cellular proteins, and these processes cannot be excluded as potentially important mediators of toxic response. Various epoxide derivatives of POM have been tested for their ability to bind to nucleic acids, and it appears that guanosine residues may be the preferred targets for reactive arene oxides. Reactivity with nucleic acids and synthetic polynucleotides (poly G) varied with the position of the epoxide moiety; K-region and non-K-region epoxides were capable of extensive covalent binding. It was further shown that, for BaP, the binding to RNA occurring after *in vivo* exposures may be due to the reaction of BaP 7,8-diol-9,10-epoxides with the 2-amino group of guanosine. A DMBA diol epoxide was also shown to be involved in DNA binding in cultured mouse embryo cells. These studies have helped to strengthen the concept that: (1) reaction with nucleic acids may be the molecular basis by which a POM exerts its oncologic effects, and (2) non-K-region diol epoxide metabolites may be the ultimate reactive chemical forms of POM.

6.9.3 Toxicology

Not a great deal of attention has been paid to the noncarcinogenic effects of exposure to POM. Nevertheless, it is known that tissues of the rapidly proliferating type (e.g., intestinal epithelium, bone marrow, lymphoid organs, testis) seem to be preferred targets for POM-induced cytotoxicity. This action is probably due to a specific attack on DNA of cells in the S phase of the mitotic cycle.

Acute and chronic exposure to various carcinogenic POM has resulted in selective destruction of hematopoietic and lymphoid elements, ovotoxicity and anti-spermatogenic effects, adrenal necrosis, and changes in the intestinal and respiratory epithelia. For the most part, however, tissue damage occurs at dose levels that would also be expected to induce carcinomas, and thus the threat of malignancy predominates in evaluating POM toxicity. For POM which are not carcinogenic, very little seems to be known concerning their involvement in toxic responses.

Immunosuppression by exposure to POM has been reported, although it is not clear whether this effect may be involved in carcinogenesis. However, it appears that the degree of immunosuppression (cell-mediated and humoral) by POM is correlated with carcinogenic potency. On the other hand, a dissociation between carcinogenic and immunosuppresive effects can be shown, whereby doses which are insufficient to affect immunity can still induce tumors.

One of the most toxicologically significant processes involved in the response to POM absorption is the interaction with drug-metabolizing enzyme systems. The induction of this enzyme activity in various body tissues by substrate and non-substrate xenobiotics may be a critical determinant in the generation of reactive POM metabolites at the target site for tumor induction. Recent emphasis has been placed on determining the drug-metabolizing capacity of humans as a measure of their potential to form activated POM metabolites. Although it has not thus far been possible to definitely correlate enzyme activity in humans with susceptibility to carcinogenesis, it is known that wide variations occur in human carcinogen-metabolizing capacity. Moreover, tissue-specific enzyme inducibility may affect the response of different organs to carcinogen action. Nevertheless, the obligatory coupling of metabolic activation with POM-induced neoplasia in animals indicates that the modulation of drug-metabolizing enzymes in humans plays a central role in carcinogenesis.

6.9.4 Mutagenesis

The demonstration of mutagenic effect in bacterial and mammalian cells by exposure to POM is generally equated with the capability to induce tumor formation.

This assumption is based on the participation of a common electrophilic metabolite in producing the carcinogenic/mutagenic event, and the common target site in the cell (i.e., DNA or other components of the genome) for the effect to be produced.

In recent years, considerable research effort has been directed at determining the mutagenicity of various POM derivatives as a means of identifying structural features associated with the biological effect produced. Working with bacterial mutants which can be reverted to histidine independence by a chemically-induced mutation, epoxides of carcinogenic POM were shown to possess significant mutagenicity. In particular, it was found that a non-K-region 7,8-diol-9,10-epoxide of BaP possesses the highest mutagenic activity of all its possible oxidative metabolites.

Further work with cultured mammalian cells established that carcinogenic POM can produce forward mutations when a drug-metabolizing enzyme system is available. Once again, a 7,8-diol-9,10-epoxide of BaP displayed the highest mutagenicity among its various metabolites. This effect was seen in the absence of drug-metabolizing enzymes, strongly suggesting that the diol epoxide derivative is an ultimate mutagenic agent. Additional investigations on the interaction of BaP derivatives with constituents of the same mammalian cells in which mutations are induced revealed that a BaP 7,8-diol-9,10-epoxide was involved in binding to DNA. The link between carcinogenicity and mutagenicity was strengthened by the demonstration that both neoplastic transformation and mutagenesis could be induced by BaP or BaP 7,8-dihydrodiol (precursor of the 7,8-diol-9,10-epoxide) in the same normal diploid hamster embryo cells.

Numerous attempts have been made to correlate exposure to POM with the induction of chromosomal aberrations. Although variations in chromosome number and structure accompany POM-induced tumors in rodents, it is not clear whether these changes are consistently observable. No evidence in the published literature has been found to indicate that POM may produce somatic mutations in the absence of neoplastic transformation.

Mutations in germinal tissues induced by POM have been more easily demonstrated in Drosophila than in mammals. The male dominant lethal assay in mice has produced conflicting results, although it is known that BaP and MCA can induce sperm abnormalities.

6.9.5 Carcinogenesis

Polycyclic aromatic hydrocarbons were the first compounds ever shown to be associated with carcinogenesis. To this day, carcinogenic POM are still distinguished by several unique features: (1) several of the POM are among the potent carcinogens known to exist, producing tumors by single exposures to microgram quantities; (2) they act both at the site of application and at organs distant to the site of absorption; and (3) their effects have been demonstrated in nearly every tissue and species tested, regardless of the route of administration. Among the more common POM at least one, BaP, is ubiquitous in the environment and produces tumors in animals which resemble human carcinomas. The demonstration that organic extracts of particulate air pollutants are carcinogenic to animals has raised concern over the involvement of POM in human cancer formation.

Oral administration of POM to rodents can result in tumors of the forestomach, mammary gland, ovary, lung, liver, and lymphoid and hematopoietic tissues. Exposure to POM by inhalation or intratracheal instillation can also be an effective means of producing tumors of the respiratory tract using very small doses of chemical. However, for both oral and intratracheal routes of administration, BaP is less effective than other POM (e.g., DMBA, MCA, DBcgC) in producing carcinomas. On the other hand, BaP has a remarkable potency for the induction of skin tumors in mice that cannot be matched by any other environmental POM. Thus, caution must be exercised in considering the carcinogenicity of POM as a class, or in using BaP as a representative example in evaluating carcinogenic risk of POM.

The induction of skin cancer by POM is regarded to be a two-stage process, involving an irreversible initiation step and requiring the subsequent presence of a promoting agent for tumors to develop. Certain POM may act only as initiating agents (e.g., DBacA, chrysene, benz[a]anthracene), or may supply both initiating and promoting stimuli (e.g., BaP, DMBA, MCA, DBahA, DBcgC, DBahP, DBaiP). The administration of a single subcarcinogenic dose of a POM (e.g., 100 nmoles of BaP) followed by repeated application of a noncarcinogenic promoting agent (e.g., croton oil) can cause the appearance of numerous skin tumors in mice. The two-stage mechanism of carcinogenesis may also apply to nonepidermal tissues.

For the induction of respiratory tumors by POM, several species and modes of carcinogen administration are employed. In studies conducted with the Syrian golden hamster, intratracheal instillation of POM established that dose-related increases in tumor yield are clearly evident, and the co-administration of carrier particles such as Fe_2O_3 can markedly increase tumor incidence.

Several _in vitro_ procedures hold promise as useful screening tools for the detection of environmental carcinogens, including POM. Morphological transformation of cultured mammalian cells has proven to be a reliable indicator of carcinogenicity by a chemical _in vivo_. Examination of DNA repair synthesis in cultured human and animal cells exposed to a carcinogen is also predictive of _in vivo_ carcinogenicity for certain compounds. When combined with the results of microbial mutagenicity tests, cell transformation assays are capable of accurately distinguishing nearly all known carcinogens from noncarcinogens.

An analysis of dose-response relationships for POM-induced tumors in animals raises several important points having relevance to environmental risk assessment. A fundamental relationship which must be considered is the deviation from linearity in dose-response curves, especially at low doses, and whether this indicates the existence of a threshold level. It can be argued that individual variability in thresholds for tumor induction resulting from saturation of detoxification mechanisms may account for a convex curvature in the dose-response curve in the low dose range. Once the dose exceeds threshold levels, tumor yield should remain a linear function of dose, with the slope of the dose-response curve being indicative of the animal's sensitivity to the carcinogen. Thus, the extrapolation of the straight portion of the dose-response curve for groups of animals is often regarded to provide a conservatively low estimate of the average threshold for tumor induction, assuming the most likely case of decreasing sensitivity with an increasing threshold. However, the use of an average threshold as a parameter of safety would not indicate the proportion of individuals still at risk of tumorigenesis at extrapolated low dose levels. Furthermore, the existence of a threshold for POM-induced carcinogenesis has not been documented in either animals or man, and, in fact, it is more likely that in diverse populations the effect of POM will be a continuous function of dose. In support of this contention are data which indicate that, in the two-stage model, tumor initiation with BaP is consistent with a linear non-threshold pattern. Overt tumor induction, on the other hand, follows a dose-response relationship consistent with a multi-hit promotion process. It is conceivable that in human populations, the multi-hit component of carcinogenesis may be supplied by environmental stimuli not necessarily linked or related to POM exposure.

The well-documented existence of cocarcinogenic and anticarcinogenic agents dictates that only a multifactorial analysis can provide a true assessment of carcinogenic risk to humans for a particular POM. Although noncarcinogenic POM were reported to antagonize the effects of carcinogenic POM's in animals, others have shown that they have little influence on tumor incidence or yield. On the other hand, several noncarcinogenic POM found in cigarette smoke (pyrene, fluoranthene, BeP) have potent cocarcinogenic acitivity. A significant decrease in carcinogenicity can be achieved with antioxidant food additives, certain vitamins, and other naturally occurring components in the diet in the experimental setting. The molecular basis of anticarcinogenesis cannot be fully explained, but may be due, at least in part, to an effect on the production of activated POM metabolites.

6.9.6 Reproduction and Teratology

Little information is presently available to indicate whether POM present a significant hazard to reproductive success. Furthermore, effects on the fetus which may be due to maternal toxicity or experimental conditions (e.g., injection vehicle, stress) have not been adequately dissociated form true embryotoxicity or teratogenesis. In cases where teratogenic effects are clearly evident (e.g., with DMBA), the required doses are far in excess of realistic environmental exposures.

6.9.7 Human Studies

The presence of POM in the air, or as components of soot, tars, and oils, have long been associated with an excess incidence of cancer in human populations. However, it has never been possible to study a population having exposure to POM in the absence of other potential carcinogens, cocarcinogens, tumor initiators, or tumor promoters.

Convincing evidence indicates an excess in lung cancer mortality among workers exposed to large amounts of coal gas, tars, and coke oven emissions. In such cases, cancer mortality can be correlated with both the type and duration of exposure. Although occupational exposure to POM-containing substances is far greater than would occur in most communities, our understanding of chemical carcinogenesis would lead to the conclusion that the number of cancers produced is directly proportional to the dose received. One must assume, therefore, that the smaller amounts of POM in ambient air contribute in some degree to the observed incidence of lung cancer in most populations. It should be recognized, however, that the influence of cigarette smoking may have an overriding impact on the evaluation of the carcinogenic threat of POM in occupational or environmental settings.

Nevertheless, an approximately two-fold excess of lung cancer occurs in urban settings as compared to rural environments, which generally cannot be accounted for by differences in cigarette smoking, age, or sex. Several investigators have shown that the incidence of lung cancer is highest in cities where POM pollutants are the most concentrated. Moreover, it is clear that among groups which have migrated from one country to another, the influence of pollution exposure in their former residence is still expressed as a higher rate of lung cancer mortality while in their adopted country. This observation cannot be explained on the basis of differences in cigarette smoking habits.

Sampling studies from different communities have attempted to separate the various factors which may be contributing to lung cancer excesses in urban settings. These studies have not provided the kind of definitive results which have been achieved with homogeneous worker populations exposed to pollutants which are well-defined both quantitatively and qualitatively. On the one hand, it was shown that lung cancer can be correlated with the extent of solid fuel combustion, and particularly BaP concentration in air. On the other hand, it has been concluded that POM in community air does little to contribute to lung cancer incidence except insofar as it may interact, synergistically or additively, with the effects of cigarette smoking.

A fundamental problem in relating exposure to POM with the incidence of cancer may involve the use of BaP levels as an indicator of total exposure. Demonstrated variation in the sources and degree of BaP emission to the atmosphere with respect to the levels of other POM precludes the accurate estimation of body burdens. In addition, animals exposed to basic and aromatic neutral subfractions of organic air pollutants commonly develop tumors, indicating that several highly active POM-type carcinogens are present in polluted air. Moreover, the carcinogenic and mutagenic activity of organic extracts of air pollutants cannot be accounted for solely on the basis of the amount of BaP contained in them. Further studies which indicate that BaP is a less effective carcinogen for the respiratory tissues than other POM (e.g., DBcgC) may thus lead to an underestimation of risks derived from data concerning BaP.

6.9.8 Ecological Effects

Although POM are found nearly everywhere in man's environment, it is not clear whether these agents may affect the ecological balance. Adverse effects on plants, microorganisms, fish, or other wildlife cannot be clearly shown. Moreover, there are limited data to indicate that POM may bioaccumulate in any species, or that transfer of POM through the food chain may occur. Indeed, animal studies showing that POM are rapidly metabolized and excreted support the contention that biomagnification is an unlikely possibility in higher organisms.

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